LEGUME-PHOSPHORUS SYNERGIES IN MOUNTAIN AGROECOSYSTEMS: FIELD NUTRIENT BALANCES, SOIL FERTILITY GRADIENTS, AND EFFECTS ON LEGUME ATTRIBUTES AND NUTRIENT CYCLING IN THE BOLIVIAN ANDES

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LEGUMES-PHOSPHORUS SYNERGIES IN MOUNTAIN AGROECOSYSTEMS: FIELD NUTRIENT BALANCES AND IMPACTS FROM SOIL TYPE AND PHOSPHORUS FERTILITY ON LEGUME ATTRIBUTES AND NUTRIENT CYCLING IN THE BOLIVIAN ANDES

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Understanding fertility management and the potential role of legumes in smallholder agroecosystems assists in promoting sustainable intensification of these systems. We used field-level nutrient mass balances in Bolivian mountain crop-livestock systems to understand drivers of nutrient cycling and gradients in soil fertility created by these drivers. Experiments with legumes and phosphorus (P) fertilization tested the response of legume attributes like nitrogen (N) fixation and residue quality to these gradients, with and without P fertilization. Legumes were fertilized with Bolivian Capinota rock phosphate and soluble P to assess soil conditions where added P would improve legume impacts.

Field nutrient balances showed that manuring rates, rangeland productivity, and soil erosion were dominant drivers of soil nutrient trends. Fields near to communities received more manure and were less steep than far fields, resulting in more positive near field balances. Mean potassium (K) trends were negative due to tuber crop harvests and export of crop residues as forage.

Across 17 experiment fields, four principal components encompassed 87% of site soil variation: P fertility and pH; organic matter; texture; and calcium phosphate (Ca-P) levels/ elevation. Phosphorus fertility was higher in near than far fields,

mirroring near/far contrasts in nutrient balances. In the experiment, legumes differed in adaptation to elevation and soil type. However P fertility was the strongest driver of legume attributes. Legumes at P-fertile sites were more likely to improve soil nutrient cycling via attributes such as N fixed, soil cover, and microbial symbioses. Legume attributes can thus mediate degrading and restoring feedbacks to soil fertility. For N, P, biomass carbon stocks, and residue quality, legumes and forage oats (a benchmark non-N fixer) had complementary attributes for soil nutrient cycling. Soil texture and Ca-P levels determined impacts of P fertilization: the largest increases in N fixed were 67% for RP and 150% for TSP in soils with low levels of clay and Ca-P. Results suggest that erosion prevention, legume-grass mixes, and P fertilization giving attention to differences in soil type and elevation, would dramatically improve sustainability of nutrient management in extensive mountain agroecosystems.

BIOGRAPHICAL SKETCH

Steven Vanek was born and raised in Ithaca, NY, but also lived abroad for periods with his family and siblings in the Netherlands, Chile, and Peru. He studied physics and secondary school education at Wesleyan in Middletown, Connecticut and then at Cornell University. He then taught environmental education and practical aspects of rural development in the Meadowcreek Center in Arkansas and the Southern Institute for Appropriate Technology in Alabama. He then volunteered with a Bolivian NGO working on aspects of integrated rural development including potable water, sanitation, and forestation. During this time he was nurturing a growing interest in agriculture and a fascination with the Andean region. This led him to a Masters of Horticulture program at Cornell University with Professor Chris Wien, where he completed a masters project testing the intercropping of lana vetch and rye between rows of pumpkins on organic farms. He then worked for two years with the Northeast Organic Network, carrying out on-farm sampling and helping to write a number of case studies of expert organic farmers in the region. He then returned to pursue a dissertation with Professor Laurie Drinkwater at Cornell University. This dissertation has been the culmination of both his fascination with mountain agroecosystems and an interest in how the impact of soil management innovations can be understood across gradients generated by soil environmental factors and by farmer management. He is married to Leia Raphaelidis and has two lively and curious children, Anaïs and Jan.

For Leia, Anaïs, Janko, and the people of Northern Potosí

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

% Ndfa	% nitrogen derived from atmosphere (fixed)
%DM	% dry matter
AM	Arbuscular mycorrhizae
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
Ap	A plaggen soil horizon resulting from human cultivation and amendment
ASTER	Advanced Spaceborne Thermal Emission and Reflection Radiometer
Bicarb-P_{org}	Soil bicarbonate-extractable organic phosphorus
BNF	Biological nitrogen fixation
C	Carbon
C:N	Carbon: nitrogen ratio
C:P	Carbon: phosphorus ratio
Ca-P	Calcium phosphate pools in soil
DEM	Digital elevation model
$\delta^{15}N$	Deviation of the ^{15}N:^{14}N ratio from that in the atmosphere in per mil units
Δh	Change in elevation of the soil surface
$\Delta h_{corrected}$	Change in elevation of soil surface resulting from denudation and corrected for settling and changes in density.
DHCl-P_i	Dilute hydrochloric acid (1 molar) extractable phosphorus in soil.
Δm_{rock}	Difference in the mass of rock between initial and final samples Of soil
E	Erosion amount in mass of soil eroded per area per year
FAO	Food and Agriculture Organization of the United Nations
H	Farmer-designated high fertility soil or field
HCl	Hydrochloric acid
ICARDA	International Center for Agricultural Research in Dry Areas
K	Potassium
K_{exch}	Exchangeable potassium in soils using the ammonium acetate extraction
KOH	Potassium hydroxide
L	Farmer-designated low fertility soil or field
LS	Topographic factor of the Revised Universal Soil Loss Equation reflecting slope length (L) and steepness (S)
masl	Meters above sea level
MEP	Micro-economic paradigm
M_{local}	Local erosion constant reflecting constants R (erodibility of soil), K (erosivity of rainfall), C (cover factor of vegetation), and P (tillage and other practices factor) of the Revised Universal Soil Loss Equation, found by regression

MODIS	Moderate Resolution Imaging Spectroradiometer: remote-sensed data of ecosystem and land processes
m_{rock-f}	Mass of rock in the final soil sample
m_{rock-I}	Mass of rock in the initial soil sample
m_{soil}	Mass of soil only, without associated rock fragments > 2mm
N	Nitrogen
N:P	Nitrogen: phosphorus ratio
$N:P_{inorg}$	Nitrogen: extractable inorganic phosphorus ratio in soils
Ndfa	Amount of nitrogen derived from the atmosphere
Ndfs	Amount of nitrogen derived from the soil
NPP	Net Primary Productivity
NRC	National Research Council
NRCS	Natural Resource Conservation Service
N_{total}	Total N assimilated from soil and atmosphere into all biomass fractions
$OH\text{-}P_{inorg}$	0.1 molar sodium hydroxide extractable inorganic phosphorus from soil
$OH\text{-}P_{org}$	0.1 molar sodium hydroxide extractable organic phosphorus from soil
$Olsen\text{-}P_i$	0.5 molar bicarbonate-extractable phosphorus from soil
P	Phosphorus
PC	Principal component or rotated factor derived from a principal component
PCA	Principal Components Analysis
POM	Particulate Organic Matter
r_b	Bulk density of soil
rb_{-f}	Bulk density in the final sampling
$r_{b-f,\ soil}$	Bulk density of soil portion only in the final sampling
$r_{b-f,\ std}$	Bulk density in the final sampling, standardized to the content of small stones in the initial soil sampling
$r_{b-f,corrected}$	Bulk density in the final sampling, corrected for lowering of the soil surface.
r_{b-i}	Bulk density in the initial sampling
$r_{b-subsoil}$	Bulk density of subsoil immediately below the sampled depth
RP	Rock phosphate
RUSLE	Revised Universal Soil Loss Equation
S	Ratio of corrected final to initial bulk density of soils
SEFO	*Semillas forrajeras*, Cochabamba-based forage seed company associated with the San Simon University.
SOM	Soil organic matter
TSP	Triple-superphosphate phosphorus fertilizer
$V_{tot-\ f}$	Total volume of cores for soil sample at final sampling

INTRODUCTION: TOWARDS AGROECOLOGICAL INTENSIFICATION IN MOUNTAIN AGROECOSYSTEMS

In extensive mixed smallholder farming systems in the Andes, productivity of the agro-ecosystem is strongly linked to food security. The area's rugged topography, low population density, and distance from markets give local food production primary importance in food availability. Purchased inputs such as fertilizer are by and large not used, and cropping places heavy reliance on locally harvested manure from rangelands and regeneration of soils during fallow phases in the rotation. Soil fertility is managed at levels that are nearer to deficiency than to the production-driven optima maintained in industrial agriculture, and it is thus likely that crop plants in the low-input Andean system are making greater use of ecological services from plant-microbial symbioses such as arbuscular mycorrhizae and rhizobia, and depend more heavily on soil ecological conditions to foster the best possible productivity.

In light of the strong link between agroecosystem function and food security and the likely nutrient limitations in these systems, a trend towards shortened fallow lengths and degradation of soil resources is troubling. Greater tillage and cropping intensity would be expected to result in reduced organic matter and increased erosion losses on hillside fields. This unsustainable intensification of the cropping system would thus result in a downward spiral of degradation of local natural resources and with lowered crop and rangeland productivity.

Because these are mountain agroecosystems, trends towards degradation are superimposed on a number of different gradients taken up in our research. Environmental gradients are those such as elevation, differences in climate, and soil pH. Soil type heterogeneity is another part of these environmental gradients, with variation in soil characteristics such as texture that relate to differences in parent

material and soil genesis. Meanwhile management gradients also exist, related to differences in manure application and cropping intensity, which is closely associated with the length of fallow.

To foster a more sustainable intensification reliant on agroecological processes that can be managed locally by farmers, our research focused on legume attributes and symbioses. We hypothesized that legumes and biological N fixation could make important contributions to such an agroecological intensification approach, and that it was important to know where challenges and opportunities will arise in the use of legumes along the different environmental and management gradients in the cropping system. The dissertation thus was an attempt to first define the nature and extent of fertility gradients created by management in these cropping systems, and then define how legume attributes and symbioses function along these gradients in processes that help maintain or restore productivity.

In Chapter 3, we present the results of research on the spatial extent, key macronutrients, and factors that drive gradients in soil fertility in these systems. Communities with lower rangeland productivities also had lower manure application rates, a result that suggests that rangeland degradation might be affecting crop production and food security through reduced manure availability. Phosphorus (P) application rates were lower in far than in near fields, and far fields were steeper so that it is difficult to replace substantial erosion P losses in far fields with application of manure. Of the three crop nutrients nitrogen (N), P, and potassium (K), mass balances in chapter four suggest that soil P gradients from near to far fields are functionally the most important one created by management. Strong contrasts in P trends between near and far field balances are consistent with the higher P levels in near fields reported in our experimental work testing legume attributes and response to rock phosphate (Chapter 1). The magnitude of erosion losses on steep fields also suggests

that erosion management is a key way to improve the nutrient efficiency and eventually, productivity of the system, a point highlighted in the scenarios for intensification in Chapter 3.

In Chapters 1 and 2, we report on the response of legume attributes and symbioses to these gradients in soil fertility and also the other site factor gradients such as soil pH, soil texture, and site temperature inherent to a mountain agroecosystem. Chapter 2 presents legume response to ambient fertility along these gradients, while Chapter 1 elucidates the response of legume attributes and symbioses in these gradients to P added using soluble fertilizer and sparingly soluble rock phosphate (RP) forms. In these experiments, legume cultivars representing different species were used to bracket the range of legume species' adaptation to environments. By testing the response of such important legume attributes as N fixation to soil fertility gradients and other soil differences, the experiments also sought to probe potential impacts resulting from greater use of legumes in lieu of non-N fixing cereal crops within these gradients. At ambient fertility in Chapter 2, we found that soil P gradients are a dominant driver of rhizobial and mycorrhizal symbioses for legumes as well as a number of attributes like carbon:P ratios in residues and total N and P uptake. Comparisons between legumes and oats, a benchmark cereal crop, suggested that greater integration of legumes into crop rotations would aid in regeneration of soils when ambient soil P supplies are sufficient to allow substantial legume biomass and N fixation. For example, for the high fertility environments legumes had higher total N assimilation from soil and the atmosphere compared to oats.

When soil P supplies are low, as is often the case in outfields far from communities, Chapter 1 suggests that P addition in either form can catalyze greater contributions of legumes to N and P in food, forage and soil. We also found that soluble P forms are effective in a greater range of soils than are RP forms. We found

conditions that permit maximum benefits of RP for legumes, such as light soil texture, low pH, and low P fertility in soil. These findings represent short-term effects. As for longer-term effects that our research did not directly investigate, it is possible that soluble P fertilizer might have the effect of diminishing mycorrhizal symbioses over the longer term, and that RP might have greater residual impacts on P availability due to gradual dissolution of the primary minerals in RP.

The form of RP we used is a Bolivian form that was mined in Capinota, Bolivia, less than 100 km from our experiment sites with smallholder farmers, and represents a potential low-embodied energy fertility source for Bolivian farmers. The technology for mining and crushing rock is well developed in the Bolivian mining sector, and we hope that our research will enable the development of local P fertility sources with a better understanding of situations where it can be effective.

Our research thus centered on the role of P in enhancing both N and P nutrient availability within crop rotations of Andean smallholder famers. However, because our research addressed a wide range of factors that contextualize the role of legumes in Andean agriculture, and because we collaborated with an NGO project in direct communication with farmers, we came to acknowledge other limitations that need urgent resolution in order to transition from unsustainable to sustainable intensification of cropping systems. Some of these limitations arose directly with in our research, while others are more anecdotal and social but bear noting here as part of the context for our more formal research. First, our nutrient balances showed that expected benefits from reductions in soil erosion were as large as or larger than the benefits of intensifying the role of legumes in crop rotations. This leads us to suggest that legumes should be combined with grasses in forage mixes, and P added in small amounts where needed, as a way of promoting higher soil coverage and reduced erosion. It also validates work that has already been done in promoting erosion

prevention with tillage methods, physical barriers and infiltration ditches, and live barrier approaches like perennial contour hedgerows using phalaris grass. The high erosion rates we measured argue for continuation of this work.

Second, in our collaboration with the staff of the NGO World Neighbors, we confronted together the possible economic and social reasons why, in spite of visible degradation of the farming landscape, change is not more rapid towards sustainable models of intensification. The first observation is that some change is in fact happening, in the form of positively deviant farmers who are trying practices such as live barriers, infiltration ditches, stone retention walls, legume forages, and legume green manures. Strengthening the role of these innovators in the communication of knowledge from farmer to farmer would be one way to reduce erosion more broadly and foster the conditions for sustainable intensification using legumes.

Third, as many other case studies in both developing and developed country context have shown, barriers to sustainable intensification have as much to do with economics and agency of farmer households as they do with technical knowledge of paths to soil regeneration. To achieve impacts, it is necessary to build community consensus aimed at confronting erosion and rangeland degradation, by first designating these problems as a subject for community consideration between positive deviant experts, outside technical staff, and the rest of the community. For this purpose, we designed a nutrient management game that can be played in workshops with farmers attending. The game allows farmer players to understand better the concept of nutrient inputs and exports, and highlights the often unknown role of erosion in erasing the gains in soil fertility from manures or N fixation.

To foster innovation, it is equally important to design measures, with input by the community, for ways that initial investments in erosion prevention or access to legume seed can be facilitated. These would include small lending schemes managed

by the community, and market incentives for alternative marketing and trade schemes in which buyers in the city incentivize the use of more sustainable practices on farms. Of course, market-based incentives to farmers might prove especially challenging in the very remote location of Northern Potosi where the market for export to urban areas is fairly sporadic. Beyond these speculative considerations, the factors that best encourage innovation of a smallholder household towards sustainable intensification are an important research question for future work.

We therefore hope to contribute, both with the formal research in this dissertation, and with the more informal remarks here, to a more sustainable intensification of cropping systems in Northern Potosi, Bolivia. We also intend our research results to be applicable to other extensive mixed crop/livestock smallholder systems that are confronting problems of unsustainable intensification.

CHAPTER 1

CONSTRAINING LEGUMES' VIRTUOUS CYCLES: SOIL TYPE AND PHOSPHORUS ADDITION IMPACTS ON LEGUMES IN AN ANDEAN AGROECOSYSTEM

Abstract

Phosphorus (P) addition has potential to enhance nitrogen (N) fixation and other legume functions in smallholder agriculture. Research can help to assess the impact of P addition across soil gradients in smallholder agroecosystems, linking farmers' soil knowledge to soil properties and testing theories of nutrient limitation and legume-symbiont interactions. In high Andean valleys in Potosí, Bolivia, we measured impacts of rock phosphate (RP) and soluble P addition on legume performance and symbioses across agroecosystem gradients in soil fertility, elevation, and soil type. We also tested correspondence between soil properties and farmer designation of 17 experiment fields as high (H) or low (L) fertility, using multivariate statistical techniques. Two legumes (*Vicia dasycarpa* and *Lupinus mutabilis*) were fertilized with soluble P (TSP) and RP in these fields. Farmer H/L designation and principal components analysis (PCA) were used to understand which soil properties best predicted impacts of P addition on legume function. PCA extracted four components (PCs) of variation among fields: management-driven P fertility, soil organic matter, soil texture, and site elevation/dilute HCl-extractable P. The first PC summarized four of five labile soil P pools and corresponded to farmer H/L designations. Neither the farmer designation nor soil P fertility of experiment fields predicted differential impacts of added P on legume biomass, P assimilation, or N

fixation. Instead, soil texture and dilute HCl-extractable P ($DHCl\text{-}P_i$) altered the impacts of added P on legume performance, with the largest impacts of P addition in soils with low clay content and $DHCl\text{-}P_i$. In these favorable settings, the N fixed by legumes was increased by 67% and 150% for RP and TSP, respectively, with similar increases in biomass and P assimilation. In fields with low P fertility, P addition narrowed C:P ratios of shoot and root of residues by between 13% and 35%. Percent cover of soil at midseason increased from 35% in the control to 45% and 55% with RP and TSP. At field sites low in soil organic matter, mycorrhizal (AM) colonization was decreased by TSP but not by RP addition, supporting the trade balance model of plant-AM response to soil N and P stoichiometry. Under appropriate soil conditions legume attributes were greatly improved by P addition, which could foster virtuous cycles in soil regeneration.

Introduction

The ability of legumes to respond to added phosphorus (P) with increased biological nitrogen fixation (BNF) is a well-known principle of plant mineral nutrition (Marschner, 1995) and has been demonstrated in both laboratory and field settings in natural and agricultural systems (Chaudhary et al., 2008; Edwards et al., 2005; Gill et al., 2006; Reed et al., 2007; Romer et al., 2004; Vera-Nunez et al., 2007) Increases in BNF from P additions may have particular importance in sustaining the productivity and livelihoods of smallholder farmers around the world (Mafongoya et al., 2006; Vanlauwe et al., 2000), especially in cases where the reliance on BNF as a supporting ecological service has declined (Snapp et al., 1998). A key research goal related to this aim is identifying how the impact of P on BNF varies across gradients of climate, soil type, and management regimes of farmers.

The use of rock phosphate (RP) to enhance legume functions like BNF in

agriculture is attractive for several reasons. RPs, particularly more plant-available sedimentary types, can be accessed through a variety of mechanisms found in legumes such as rhizosphere acidification due to BNF (Perez et al., 2007), organic acid exudation (Hocking and Jeffery, 2004; Li et al., 2003), and mycorrhizal symbioses (Barea et al., 2002; Guissou et al., 1998). Because RP requires less processing than other P fertilizers, it uses less fossil fuel for manufacture and generally also has a lower cost to farmers. RP may also reduce irreversible P sorption in soil, since primary P minerals in RP are weathered in place, leading to more positive residual impacts for added RP than for soluble P (Chang and Liang, 1968; Choudhary et al., 1994; Doll et al., 1960; Drinkwater et al., 2008). However, some have argued that RP solubilization in soils is too slow or unpredictable to reliably improve crop yields (Le Mare, 1991; Smithson and Giller, 2002). Significantly, previous tests of Bolivian Capinota RP used in our work revealed only small effects on productivity of potato and maize crops (Villaroel, 1988).

Debates about RP effectiveness in smallholder agriculture indicate the need for more comprehensive testing that identifies soil scenarios where RP is an effective P source. RP use has been studied in reductionist experiments, but it is difficult to scale up these results to real world conditions (e.g. Choudhary et al., 1994; Somado et al., 2006; e.g. Weil, 2000). Furthermore, tests of RP effectiveness and P limitation of legumes in smallholder soil environments have not typically spanned gradients in soil type, climate, or management-induced soil fertility. Evaluating P additions of differing solubility in these gradients can identify situations where RP has rapid impacts on BNF and other legume attributes that enhance nutrient cycling. The extent to which RP and soluble P additions affects legume productivity within a single season also influences farmer adoption of RP or other fertilizers for legumes.

To respond to these research needs, our study had three general objectives

focused on a particular extensive Andean smallholder cropping system: (1) characterize gradients in soil management and soil type using soil parameters; (2) measure the impact of RP and soluble P on legume attributes; and (3) understand the combination of management and soil type factors that constrain effects of P addition. We expected that fertility gradients in these extensive systems would be dominated by contrasts between *infields* and *outfields*, with more fertile infields located closer to the farm household or having favorable attributes for high crop yields. Proximity of fields and favorable soil characteristics create feedbacks that stimulate greater fertility inputs by farmers and over time maintain higher fertility in closer fields. Infield/outfield distinctions are often present in farmer knowledge systems in extensive mixed cropping systems (Konde et al., 2001) including our study area in highland Bolivia (Sanchez, 2005). We expected that characterization of soil gradients could shed light on the relationship of farmer soil fertility designations to scientific soil and site parameters such as texture, pH, and elevation. Determining how technical soil properties correspond to farmer soil knowledge can facilitate extension linkages between farmers, scientists, and development professionals.

Our second objective was to measure the impact of added RP and soluble P on legumes. First, we measured direct plant responses such as P assimilation, N fixation, biomass, and soil coverage. We predicted that legume BNF would be increased by P addition, thereby increasing biomass, soil cover, and biomass N stocks to deliver direct impacts on the cropping system such as soil regeneration from residues and protein in food and forage. We also wished to measure the effects of P addition on secondary legume attributes with longer-term impacts, like residue quality which affects decomposition dynamics, and the intensity of rhizobial and mycorrhizal (AM) symbioses. In other results from these experiment sites, we analyzed variation in legume attributes without P addition, and concluded that differences in P fertility

among sites were most important in modulating both direct and secondary attributes of legumes (Vanek, Chapter 2). Substantial literature elsewhere suggests that both primary biomass and secondary residue quality impacts of P addition affect nutrient cycling in soils (Abarchi et al., 2009; Cornwell et al., 2008; Zhang et al., 2008).

Our third objective was to use the soil fertility and soil type characterization to discern which factors explain differences in effects of P addition, or in agronomic terms, constraints on the benefits of RP and soluble P addition. We expected that ambient P fertility would cause differences in the effect of added P: at infertile sites P addition would have stronger impacts on legume attributes like P assimilation and BNF than at P-fertile sites. We also expected that other soil characteristics might be important in altering the effects of P addition. In particular, soil sorption of applied P and chemical limits on dissolution of applied RP have been suggested as limitations on the impact of RP additions (Khasawneh and Doll, 1978).

These objectives are grounded in theories of plant nutrient limitation and plant resource allocation to roots and symbioses: the *microeconomic paradigm* of nutrient limitation, the functional equilibrium theory of shoot:root relations, and the trade balance model of plant-mycorrhizal relations. Regarding nutrient limitation, the classic law of the minimum (Von Liebig, 1840) , and the more recent microeconomic paradigm (MEP, Bloom et al., 1985) both predict response to P addition in P-limited conditions. The MEP argues that plants actively adapt to nutrient constraints via allocation of fixed C to nutrient acquisition, and thus are effectively limited by many nutrients simultaneously. It suggests that P addition would allow N-fixing legumes to resolve P limitation directly, then N via increases in BNF, and subsequently other nutrient limitations via investment in fixed C to greater biomass. Allocation of increased N-fixation and biomass to additional higher nutrient uptake and C fixation envisioned by the MEP suggests that residue quality (C:N, C:P) under P addition

would change little, since C, N, and P would increase proportionally. It also suggests that RP additions would have smaller effects than soluble P addition because RP carries additional C costs of mycorrhizal investment or acid exudation by roots compared to direct soluble P uptake (Costa et al., 1989; Guissou et al., 1998).

Theories of root:shoot relations in plants (Reich, 2002) also help inform the expected impacts of P addition on legumes. As P addition increases N fixation and biomass of legumes, root/shoot functional equilibrium predicts that shoot:root biomass ratios will also increase. Because harvest of most aboveground biomass for food and forage is customary among smallholders, this could mean reduced root biomass and belowground N and P stocks resulting from P application. However if root and shoot biomass both increase with added P, residue impacts from both roots and shoots would be favored. Root/shoot relations are little-studied in smallholder agriculture, and information on these relations can clarify tradeoffs between harvest and incorporation of legumes as green manures. Our experiment therefore sought to test the impact of P addition on relative biomass stocks above and belowground as primary impacts of P addition on legumes.

Arbuscular mycorrhizal (AM) symbioses are important attributes of many legumes for smallholder cropping systems, and we sought to measure their response to P addition. Johnson's *trade balance* model (2010) helps predict this response by proposing the plant-AM symbiosis can vary between parasitism and mutualism depending one soil N:P ratios and more and less mutualistic AM taxa. The model predicts lower colonization rates with added soluble P, and/or less mutualistic resource exchange between plants without observable changes in colonization. However, when we tested legume-AM colonization at ambient soil fertility across the same set of farm fields, we found that AM colonization rates did in fact vary in response to unamended N:P ratios of soil (Vanek, Chapter 2). This suggested that colonization rates for a

mycorrhizal legume would indeed decrease under soluble P addition compared to an unamended control, with no change under RP addition since AM symbioses would be one mechanism of accessing RP.

Within this theoretical framework we then tested hypotheses related to our three main objectives. We first tested correspondence between a number of standard soil parameters, including labile soil P pools, and farmer designations of high and low fertility. We hypothesized that high/low fertility designations would correspond to measures of soil labile P, and that near fields would have higher levels of labile P in an infield/outfield scheme.

We then tested the impact of P addition on primary and secondary attributes of two legume crops. We hypothesized that P additions would augment attributes important for primary impacts of legumes: biomass, soil cover, N derived from BNF, and also total N, P, and C stocks in residues. We predicted that this increase would be less for the case of RP additions where P acquisition has a greater C cost than for soluble P. Meanwhile, we predicted that C:N and C:P ratios of legume shoots and roots, an attribute central to secondary residue impacts, would be unchanged, since added P would lead to simultaneous increases in C, N, and P assimilation according to the MEP. Based on root:shoot functional equilibrium theory, we expected that P fertilization would cause legumes to increase shoot to root ratios as well as root and shoot biomass. For a mycorrhizal legume, we hypothesized that AM colonization would be equal for the zero-P control and RP addition, while declining with soluble P additions which strongly alter the soil available N:P ratio, as theorized in the trade balance model. We also contemplated an alternative result in which the legume and AM would shift towards parasitism with no change in colonization rates.

We also predicted that P addition would interact with different levels of soil P fertility, so that added P would have larger positive impacts on legumes at farmer-

designated low fertility (i.e., low P) fields than fertile, high-P fields. However, we also expected that our soil characterization might reveal other sources of variation in the impact of added P. By testing these hypotheses, we intended to map out ways that added P might enhance legume functions such as fixed N, amounts of residues, and soil cover. Improving these characteristics at P-infertile sites could help to reverse soil fertility degradation in these smallholder systems.

Materials and Methods

Hypotheses were tested in experiments over two years (2005-2006 planting seasons) at elevations ranging from 2700 to 4000 meters above sea level (masl) in the Bolivian department of Potosí (Latitude/Longitude: 66º 58'W/17º 54'S **to** 66º 15'W/18º 15'S). Average rainfall is 650mm, occurring in a rainy season from October to March (FAO, 2010). Mean growing season temperatures ranged from 9.5ºC to 18.0ºC. Soils are dominantly eutric leptosols with some eutric and dystric cambisols in fields with deeper soils (Dijkshoorn et al., 2005).

In 2005-2006, the experiment was planted in one low and two high fertility sites at three different elevations, while in 2006-2007, 14 fields in seven communities were used, with 7 field pairs described as low and high fertility soils by collaborating farmers in each community. In the presentation of results, sites were given numerical designations arranged from low to high elevation, with L and H denoting low and high fertility (Fig. 1.1). All fields were in the last year of the local crop rotation typified by potato, then fava bean or maize or small grains, then small grains. Wheat, oats, or barley was the preceding crop in all fields. Fifteen fields were on mineral soils derived from sedimentary rock (sandstone, shale) while two sites (8L and 8H) had high soil organic matter (SOM) derived from peaty substrates at 3700 masl, above which these soils are not used for growing legumes (Fig. 1.3; Table 1.1 and 1.2).

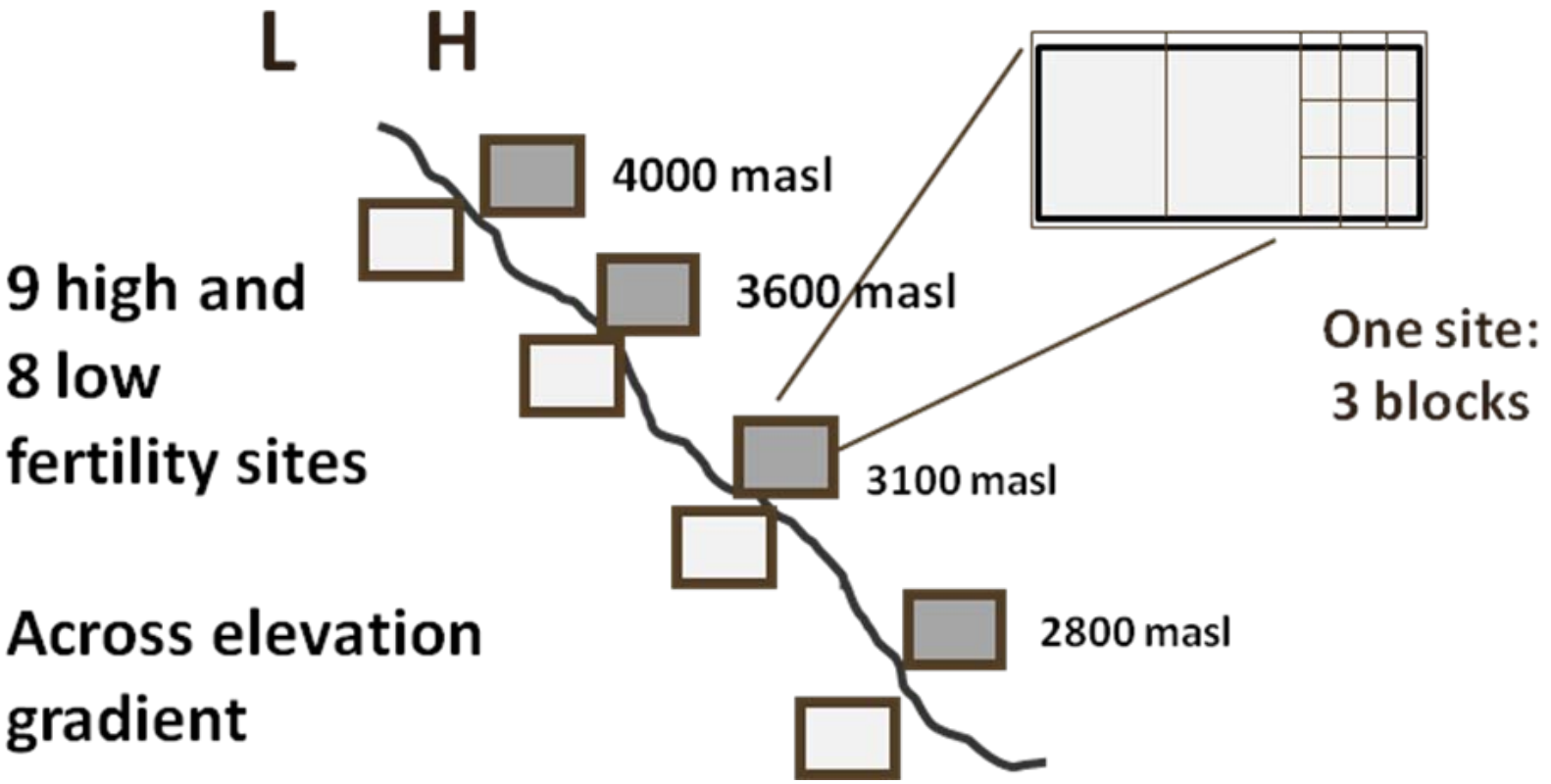


Figure 1.1. Arrangement of experimental sites and blocks along an elevation gradient and with respect to farmer-designated high (H) and low (L) soil fertility.

Soil and site characterization

For each of 50 blocks at 17 sites included in the experiment, 11 soil parameters were used to characterize site and block variation. These parameters were mean temperature for the growing season, soil pH [aqueous suspension 1:2.5 method], total C and N (by combustion, LECO, St Joseph, MI), percent sand, silt, and clay (Bouyoucos method), and five P fractions of differing availability. P fractions were measured in a sequential pool extraction modified from Tiessen and Moir (1993): bicarbonate inorganic (Olsen-P_i), bicarbonate organic (Bicarb-P_{org}), 0.5 M NaOH-inorganic and organic pools (OH-P_{inorg} and OH-P_{org}), and 1M-HCl extractable inorganic P (DHCl-P_i). We omitted resin-exchangeable P in the first extraction step so that our most labile pool is the Olsen P pool, and also more recalcitrant fractions beyond dilute HCl-P_i since these were assumed to contribute little to plant nutrition within the time-course of the experiment. Average site air temperature for the growing season depended strongly on site elevation (R^2=0.98) and we used it interchangeably with elevation in statistical analyses. In addition to these 11 soil variables, percent slope of fields, measured on profile photos of sites, and distance to each field, measured using a distance tracking function in Manifold GIS software (Manifold, www.manifold.net, Carson City, NV) were used in some analyses.

With the 11 soil variables excluding site temperature, canonical discriminant analysis (JMP, SAS institute, Cary NC) was used to query which soil variables discriminated most strongly between high (H) and low (L) fertility fields as categorized by farmers. Forward and backward stepwise variable selection (JMP, SAS institute, Cary NC USA) was first used to identify variables that ranged from statistically significant to non-significant (with $p<0.60$) in the analysis. These variables were then used in a final analysis. We also used t-tests to compare soil and site variables for groups of H vs. L fields.

To understand common variation among site variables and reduce dimensionality of soil parameters in statistical modeling, principal components analysis (JMP) was employed with the 11 soil variables given above. Besides these 11 soil parameters, rainfall and biomass of an oat reference crop in each block were measured, but only soil parameters were used in the PCA because they were free from year to year variation. In this way our analysis of results would have better predictive value for other sites in the same region. Orthogonal rotated factors were extracted from a set of the first few principal components (PCs) chosen to account for a majority of site variation and reflecting interpretable combinations of site variables. Rotated PCs (varimax rotation) were interpreted based on loadings of the individual site variables on each PC, and were used as covariates indicating site and block-level variation in the statistical analysis.

P addition impacts on legumes were tested using cultivars of two legume species with different attributes and uses, both adapted to the Bolivian Andes (Wheeler et al., 1999): The endemic Andean lupine (*Lupinus mutabilis* Sweet, local name *tarwi*) is consumed as a nutritious food after removal of bitter alkaloids (NRC, 1989). It is also used by farmers as a green manure and as an income source through sales to growing urban markets (Aguilar, personal communication). The introduced legume *Vicia dasycarpa* (lana vetch) is an annual forage crop. It can be planted with local forage oat varieties to enrich hay stockpiled for animal feed in the dry season, and was also being tested by some farmers locally as a green manure. These legumes use different strategies to augment root-acquired soil P by expending fixed C. Lana vetch is mycorrhizal and allowed us to assess changes in AM colonization across a soil fertility gradient in response to added P. Andean lupine is thought to be non-mycorrhizal and exudes organic acids for P acquisition, also forming proteoid roots but only under conditions of prolonged P starvation (Hocking and Jeffery, 2004).

The multi-level experiment design used three experimental blocks at each of 17 sites over two years, with two blocks at one site (8H) due to small field size. Experimental treatments were a species-by-P-fertilization factorial with lupine and vetch as species, and three P levels: an unfertilized control (0P) and 40 $kg \cdot ha^{-1}$ P as either rock phosphate (RP) or triple superphosphate (TSP). A weed-only and forage oat control, with legume weeds removed, were added to each block for comparison of N and P uptake by non-legumes and for use as reference plants in the 15-N natural abundance method (described below). Crops were sown in 2x2 m plots at the high end of seeding rates typical for these crops in the area (oats at 120 $kg \cdot ha^{-1}$, vetch at 90 $kg \cdot ha^{-1}$, lupine at 60 $kg \cdot ha^{-1}$). Vetch and oats were broadcast in 2005-06, and sown in rows at 15 cm spacing to facilitate weeding and root sampling at the 14 sites in 2006-07; lupine was sown in rows at 50 cm spacing in both years. Capinota RP (Universidad San Simon soil lab, Cochabamba, Bolivia) and TSP were mixed into soil with a pick prior to seeding. At sloped sites, contours were trenched between rows of adjacent upslope/downslope plots to prevent downslope movement of soil and fertilizer from fertilized plots onto unfertilized plots. Plots were weeded twice to prevent weeds from obscuring treatment effects and make root sampling relevant to the species of interest. We measured season rainfall with gauges in communities with experiment fields, and air temperature at fields using HOBO temperature loggers within shaded enclosures (Onset, Bourne, MA).

Quantification of plant biomass fractions:

Aboveground biomass was determined by cutting and weighing fresh biomass from interior rows, excluding row ends, or 1x1 m quadrats for broadcast plots in 2005-2006. Dry matter content (%DM) was found by weighing a representative chopped sample in the field of approximately 300 g weight, then air drying the sample in a brown paper bag on rooftop or other surface in strong sunlight at approx 45°C, with

finish drying in an oven at 60°C before final weighing for %DM. Taproot, crown, and nodule biomass was measured to 25 cm depth by excavating two randomly chosen rectangles of furrow width, 25 cm long for vetch and 50 cm long for lupine, on interior rows of each plot. Taproots were counted for an estimate of plant stand, with additional 25-cm lengths of rows counted, but not excavated, to improve the precision of stand estimates for vetch. All lupine stems in interior rows were counted to estimate lupine stand. Taproots, crowns, nodules and roots were kept in a cooler or refrigerated until they could be separated between taproots, nodules, and a subsample of root biomass for nutrient analysis and mycorrhizal staining. For nutrient analysis of roots, a 'cookie' containing a large number of root diameters from 2 mm to <0.5 mm was gathered from excavated roots and washed in tap water. To prevent rot and aid drying, root biomass subsamples and nodules were surface sterilized by placing in 90% ethanol before drying in coffee filters. A 15-degree sector comprised of a large number of root fragments was cut out of the root cookie before drying and placed in a 20 ml vial of root fixative (35% ethanol, 3% acetic acid) as a random sample of root segments for root staining and scoring of mycorrhizal colonization (see below).

Root biomass was estimated by bulking 16 soil sample cores in each experimental plot, eight cores taken from between rows (or at interplant positions with broadcast species in 2005-06), and eight cores taken from approximately 3cm from plant crowns, for wet sieving and recovery of roots (below). Of these, between 8 and 10 undisturbed, intact cores were initially combined in a separate container and weighed for an estimate of moist bulk density, calculating volume from depth and diameter of the core. Our root sampling ignores roots below 25cm depth, a potential shortcoming of the approach. We noted in several exploratory excavations in fields that the effective rooting zone of these mountain soils is shallow, so that most nutrient uptake is likely happening in an Ap horizon. Our method thus measured roots for

nutrient uptake in the upper soil layer where P fertilization occurred, and roots in upper horizons relevant for decomposition and availability to subsequent crops.

Root biomass samples were kept in a cooler and moved to a refrigerator at below 3°C within 36h, and processed within two weeks for estimates of fine root biomass carbon. Root biomass carbon was estimated using wet sieving and separation of roots from particulate organic matter (POM): 300 g of moist soil was soaked in tap water for 10 minutes and then decanted through 2mm and 0.5mm stacked sieves, then rewashed, gently breaking down soil aggregates by hand, until only small stones and sand remained in the wash basin, roots and light-fraction POM had been collected on sieves, and clay and silt had been washed through the sieves. POM and roots from the 2-mm sieve fraction, which contained roots down to 0.2mm diameter, were hand separated in water with forceps for the first block of each site, and with air-dried samples for blocks two and three. Roots were dried and weighed on a microbalance, and then analyzed for C and N with a LECO combustion analyzer (St. Joseph, MI). Analyses of C:N and C:P from the larger sample of roots harvested in excavations from each plot were used to back-calculate the amount of N and P associated with the C in the cored root samples.

It is reasonable to assess the rigor of dry versus wet separation of roots and POM. Although this variability is controlled for by blocking in the experimental design, we checked equivalence of the two methods by assuming equal levels of soil POM across the spatial extent of one experiment site, and testing whether POM mass recovered from wet separation in block one vs. dry separation in blocks two and three were approximately equal, using the mean and variance of plots within a block to construct an F-test. We also tested whether there was a systematic increase in measured root biomass associated with lower POM recovery between blocks at a site where dry vs. wet separation was used. Both tests showed that recovery of roots

between wet and dry separation would not introduce systematic errors in inferences related to treatment differences. For example dry separation recovered 87% (+/- 8.5% std. error) of the POM compared to wet separation, not statistically different from 100%.

The 0.5 mm sieve root fraction was not sorted between POM and root biomass. Inspection of the 0.5 mm sieve fractions on scanned images of unsorted roots and POM in trays showed that roughly 25% of the sample was small root fragments, which means that roots recovered on the 2mm sieve provided only a lower bound on the amount of root biomass. The 2mm root samples were however representative in terms of sampling different root size classes and indicating treatment and species differences on the actual root biomass.

Staining of roots and scoring of mycorrhizal colonization

Roots were cleared and stained using the methods outlined by Koske and Gemma (1989). Small subsamples from stored, fixed roots in vials were cleared in 10% KOH solution at 90ºC for 14 min. (lupine) and 16 min. (vetch), with clearing times pre-tested for best staining results. Roots were acidified in 1% HCl for 6h, stained with trypan blue stain, and then destained in acidic glycerol (>2 days) before mounting on slides. Twenty-five to thirty separate root segments were mounted per slide for microscope scoring at 400x magnification. Each slide was scanned and roots encountered in the microscope field were scored positive for AM colonization if fungal hyphae occurred along a perpendicular line traversing the root, *and* if further, this hypha or hyphae was connected to diagnostic AM mutualistic structures (arbuscules or coils) within three microscope fields of view along the root. Fifty root intersections were scored in this way for each slide, and percent colonization defined as the fraction of these that scored positive. Root staining and scoring of AM presence measures only intraradical presence of the fungi, and does not assess AM biomass in

soils or plant-AM resource exchange directly, a shortcoming acknowledged by Johnson et al. (2003). We assumed that staining and root scoring would be sensitive enough to detect only influences on legume-AM symbioses from P fertilization that were strong enough to cause plants to actively reduce infection rates

Nutrient analyses

After grinding to 30-mesh size, total C and N was measured by combustion in shoot biomass, taproot biomass, nodules, and roots harvested from excavations (LECO C and N analyzer, St. Joseph, MI). In the case of small root samples from washed cores the entire sample was combusted. Total P in the biomass fractions was determined using a nitric acid digestion of ground plant tissue with H_2O_2 addition to oxidize organic carbon in the final digestion steps (Kalra, 1998). National Institute of Standards and Technology apple leaf standards were used to verify >92% recovery of P from samples. Digested residue was dissolved in 4% HCl and analyzed for orthophosphate using the molybdate blue method on a Seal AQ2 discrete auto-analyzer (SEAL Analytical, Mequon, WI). C, N, and P in above- and below-ground fractions were calculated as:

C, N, or P stock ($kg \cdot ha^{-1}$) = biomass of fraction ($kg \cdot ha^{-1}$) * % nutrient

Estimation of %Ndfa with natural abundance of ^{15}N:

Proportion N fixed via BNF (%Ndfa) and amount fixed (Ndfa) was calculated using the natural abundance method described by Shearer and Kohl (1986). Briefly, the ^{15}N signature ($\delta^{15}N$ or deviation of ^{15}N: ^{14}N from that in the atmosphere) of a field-grown legume is assumed to result from mixing between the $\delta^{15}N$ signature of N uptake from soil, measured using non-fixing reference plants, and $\delta^{15}N$ for the legume when grown using only atmospheric N (the legume's B-value or B_{legume}). Percentage N fixed (%Ndfa) is estimated as the field-grown legume's $\delta^{15}N$ as a proportion of the distance in $\delta^{15}N$ values between 0% fixed (reference non-legume) and 100% fixed (the

B value):

$$\% \text{ Ndfa} = 100*[(\delta 15\text{N reference} - \delta^{15}\text{N legume}) / (\delta^{15}\text{N reference} - B_{legume})]$$

%Ndfa was multiplied by N uptake in each biomass fraction to give amount Ndfa in the different biomass fractions. δ^{15}N was assessed on plant samples homogenized by roller-grinding in glass jars with stainless-steel rollers. 3.5 to 4.2 mg ground plant matter was weighed into tin capsules for analysis at the University of California-Davis stable isotope facility (Davis, CA). We used aboveground biomass samples of *Cichorium intybus* and tap-rooted endemic non-legume weeds (e.g. *Bidens pilosa, Erodium spp., Tagetes spp.*) as reference plants. B-values for vetch and lupine were assessed with plants grown in sterile media (Turface proleague, Profile LLC, Buffalo Grove, IL) under greenhouse conditions with four pots of each species. Four plants of vetch and two of lupine were grown per pot and inoculated with the same inoculants used in the experiment. At flowering, aboveground biomass from each pot was cut, dried, and ground for ^{15}N analysis. The mean value of δ^{15}N for the four pots was taken as the B-value for that species.

Results:

Field soil characteristics and discriminant analysis of farmer field groupings

High (H) fertility fields as classified by farmers had higher Olsen-P_i and OH-P_{inorg} than did low-fertility (L) fields (Fig. 1.2c, t-test, p=0.003, Table 1.1). Bicarb-$P_{org,}$ OH-P_{org}, and soil %C were also higher in H than L fields when two high-organic matter fields (8H, 8L) were excluded ((Fig 1.2b, Table 1.1, t-test, $p<0.05$). Field slope and household-field distance were higher for L than for H fields (Table 1.1), suggesting infield/outfield distinctions in farmer management and higher erosion rates in far fields. Meanwhile, soil textural variables and soil pH varied widely but not significantly between H and L fields (Fig. 1.2a, Table 1.1). Dilute HCl-P_i was

generally higher in H fields at middle to high elevation, while showing contrasting results in two L fields at low elevations with high levels of Dilute HCl-P_i (Fig. 1.2d).

A discriminant analysis excluding fields with high SOM (8L, 8H) separated H and L fields (Fig. 1.3, Wilks-lambda $p<0.001$), and Olsen-P_i and total soil %C were significant in distinguishing the two classes of fields (Fig. 1.3 inset). The discriminant analysis and the field-level results above supported our hypothesis that farmer distinctions of H and L fields corresponded to measures of soil P fertility. The wide range in soil P fertility made these fields a suitable environment to test the hypothesis that impacts of P addition on legume attributes would vary with P fertility in these smallholder farming systems.

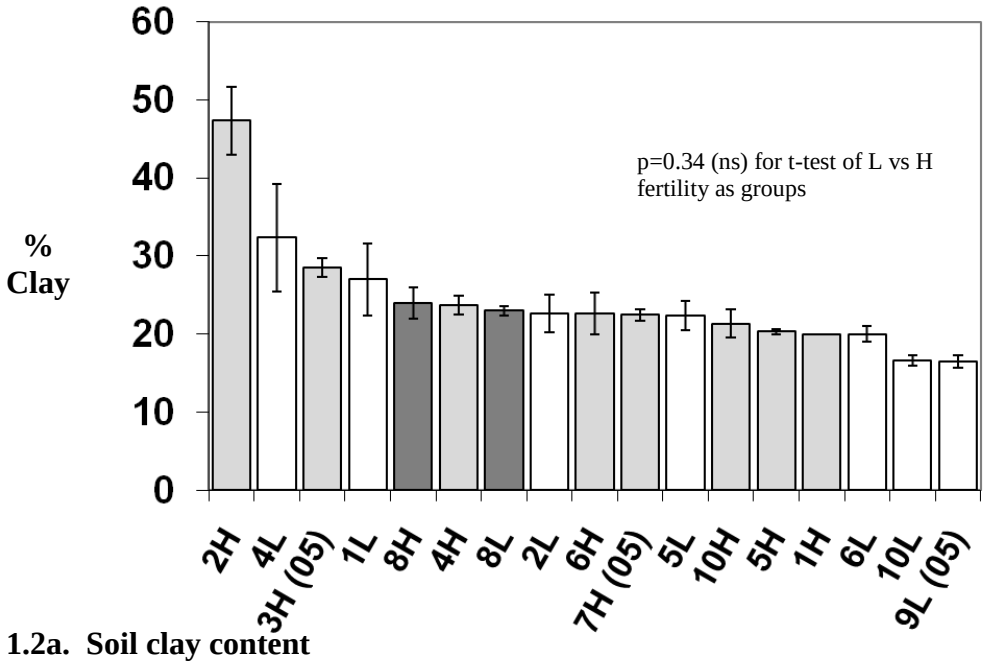
PCA on field soil characteristics*:*

Principal components results confirmed the importance of soil P fertility as a dominant gradient among fields in the experiment, and clarified relationships among other soil parameters for field sites. Because extracted rotated factors (PCs) were orthogonal, PCA also resolved problems of multicollinearity among predictors in statistical models analyzing the impacts of P addition (e.g. positive correlations among soil P fractions and negative correlation between % clay and % sand). A varimax rotation extracted four PCs with loadings of the 11 soil variables (Table 1.2). These PCs accounted for 87% of the variation among 50 blocks of the experiment and had interpretable meaning based on loadings of soil parameters.

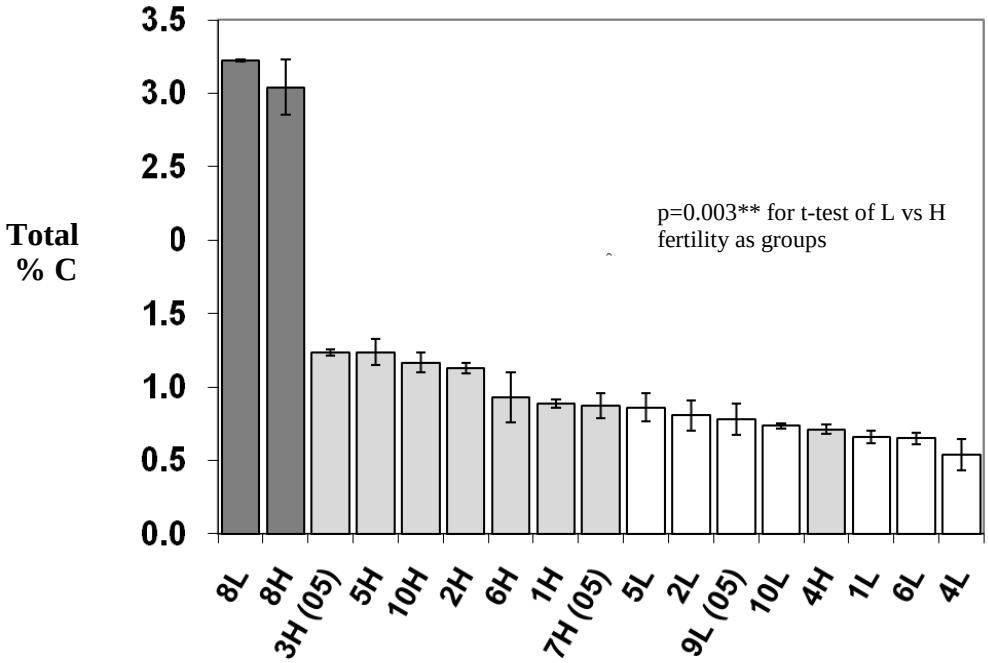
Table 1.1. Field soil properties in experiment fields not shown in Figure 2. Mean values of three blocks per site are shown. Fields were Low (L, unshaded rows) or high (H, shaded rows) fertility as designated by farmers. Significance of t-test comparing groups of L vs. H is given at bottom (* $p<0.05$; ** $p<0.01$; ns, not significant). Number order of fields shows elevations from low to high. Dark shaded 8L and 8H sites are two sites with high soil organic matter on peaty substrates

Site (numbered low to high elevation)	Site slope (%)	Distance to house-hold (m)	Bicarb-P_{org}	NaOH-P_{inorg}	NaOH-P_{org}	%Silt	%Sand	Soil total %N (g/kg)	Soil pH
1 L	27	190	36	7	18	34	39	0.79	8.3
1 H	7	110	162	57	54	32	48	0.82	6.2
2 L	12	550	80	22	45	27	50	0.75	6.4
2 H	9	20	86	33	41	18	35	1.13	7.3
3 H(05)	12	600	109	163	207	36	35	0.89	5.2
4 L	30	220	59	27	27	31	37	0.67	6.7
4H	7	30	140	73	52	41	35	0.68	5.7
5 L	32	325	132	29	53	35	43	0.96	5.2
5 H	7	30	215	70	95	27	52	1.08	5.1
6L	22	550	155	40	64	32	48	0.74	5.5
6H	9	50	281	79	56	34	43	0.75	5.0
7H (05)	9	30	99	77	105	30	47	0.72	4.7
9L (05)	9	170	14	24	53	35	49	0.83	5.8
10 L	7	150	112	24	33	34	49	0.89	6.0
10 H	9	20	145	27	49	37	42	1.06	6.0
8 L	27	150	451	129	303	35	41	1.90	4.7
8H	9	10	544	77	253	29	48	2.24	5.1
Mean of L fields (std error):	**21** (4)	**288** (60)	**130** (49)	**38** (13)	**74** (33)	**33** (1)	**44** (2)	**0.94** (0.14)	**6.1**
Mean of H fields (Std error):	**9** (1)	**100** (63)	**198** (48)	**73** (13)	**101** (26)	**32** (2)	**43** (2)	**1.04** (0.16)	**5.6**
P value for t-test L vs. H fertility	**	**	ns♣	*	ns♣	ns	ns	ns	ns

♣ Significant at $p<0.05$ when 8L and 8H sites with high organic matter are removed.

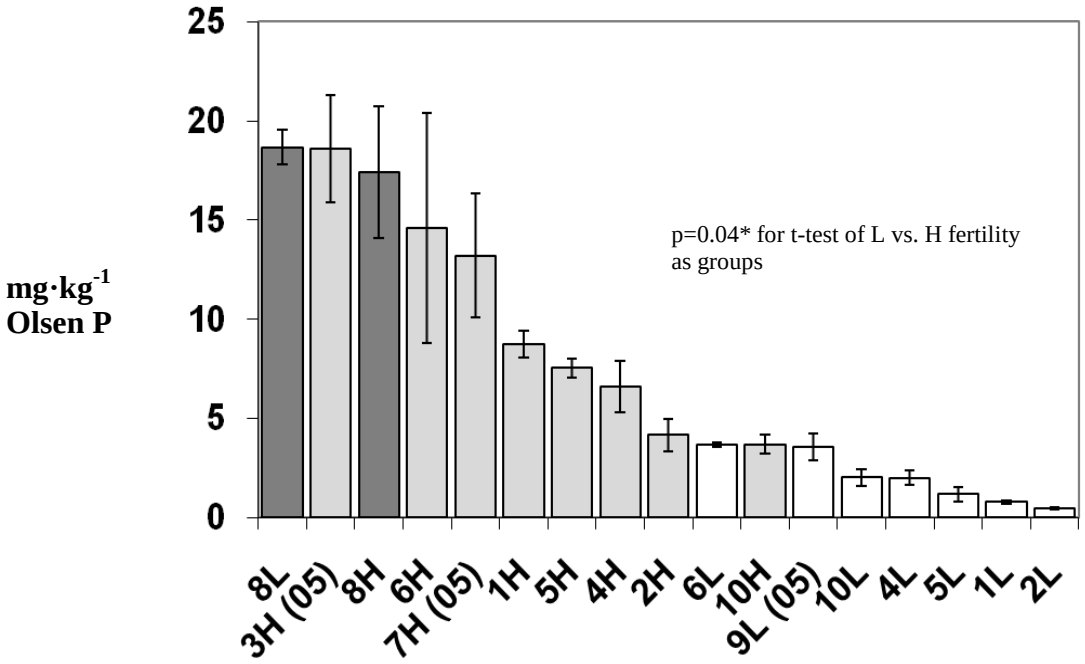


1.2a. Soil clay content

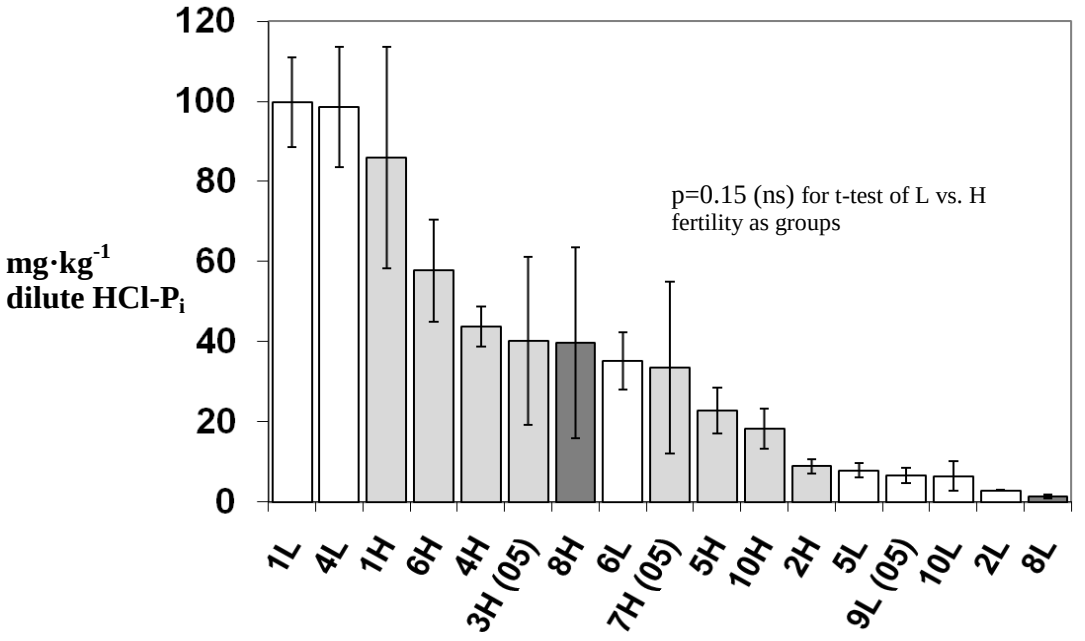


1.2b. Total soil C content

Figure 1.2a,b. Site means and standard errors of selected field soil characteristics used in the discriminant and PCA analysis. Dark bars are high fertility fields (H) as classified by farmers, light bars are low fertility (L); 8L and 8H are less and more productive soils designated by farmers with high organic matter. Sites marked (05) are from the 2005-06 season; the remainder were used in 2006-2007.



1.2c. Olsen-extractable P_{inorg}



1.2d. Tiessen dilute HCl-P_i

Figure 1.2c,d. Site means and standard errors of selected field soil characteristics used in the discriminant and PCA analysis. Dark bars are high fertility fields (H) as classified by farmers, light bars are low fertility (L); 8L and 8H are less and more productive soils designated by farmers with high organic matter. Sites marked (05) are from the 2005-06 season; the remainder were used in 2006-2007.

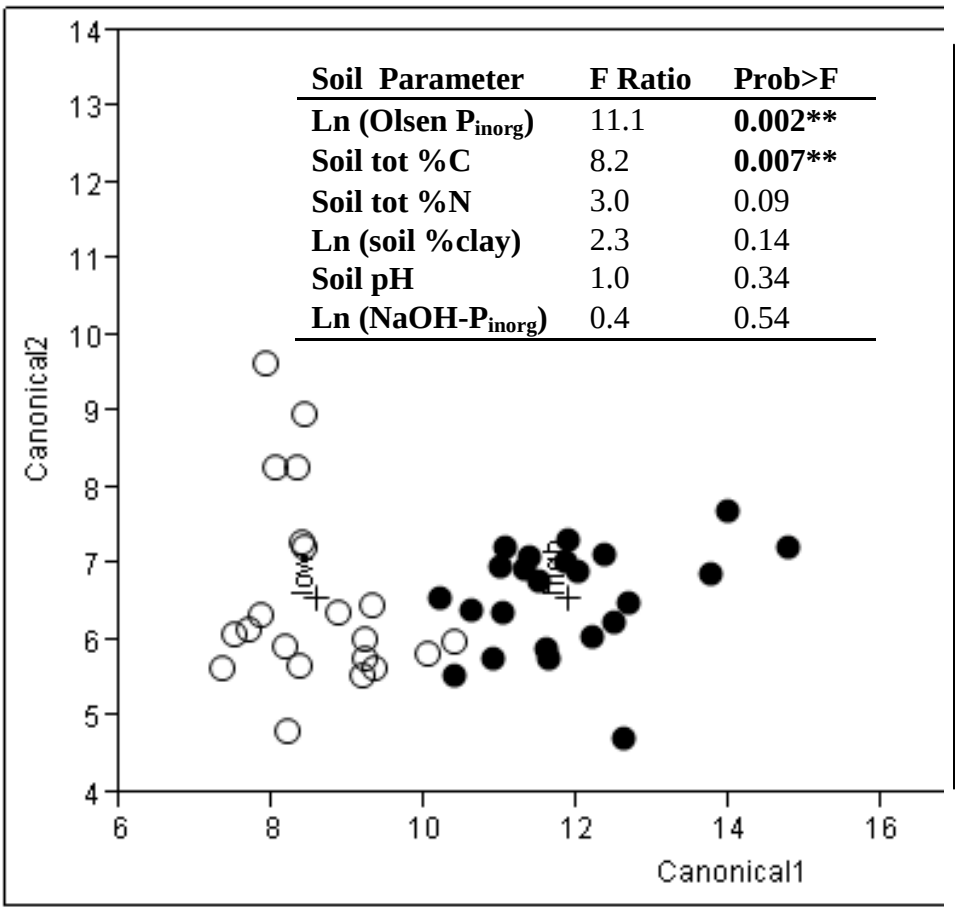


Figure 1.3. **Score plot from discriminant analysis of fields designated as high fertility (filled symbols) or low fertility (open symbols) by farmers**. The Wilks lambda test for separation of sites was significant at $p<0.0001$. Table inset shows F ratio and significance of seven soil variables in the corresponding discriminant analysis using farmer-selection of fields as high or low fertility as a category.

Principal component 1 (PC1) summarized variation in all the sequentially extracted P pools except dilute HCl-P_i (Table 1.2), and was also correlated to P uptake by the forage oat reference crop planted in each block ($R=0.59$, $p<0.001$). Soil pH was inversely correlated with these P pools on PC1, reflecting acidity from two likely sources in more P-fertile middle and high elevation sites: more positive hydric balance causing greater soil leaching (seen in the negative site temperature loading for PC1), and pH buffering from higher organic matter (seen in the positive soil C content loading). PC1 distinguished farmer soil fertility categories, with PC1 scores of high and low fertility fields significantly different (t-test, $p=0.015$). This separation is seen in the PC1 by PC2 biplot (Fig. 1.4), where H and L fields segregate into two clusters along PC1. Separation along PC1 is consistent with the discriminant analysis presented above (Fig. 1.3) and management-induced differences in inorganic P pools between H and L fields (Table 1.1, Fig. 1.2).

Principal component 2 (PC2) loaded measures of soil organic matter: soil total C and N content and bicarb-P_{org} from the sequential P extraction. Because of high soil C content and bicarb-P_{org} levels, the 8L and 8H sites were distinct from other sites along PC2, although they were not outliers when multivariate distance (Mahalonobis distance) was assessed in the PCA. PC2 was correlated to N uptake by the reference oat crop in each block and was thus related to soil N fertility ($R=0.40$, $p=0.02$). Unlike for PC1, H and L fields did not segregate along PC2 (Fig. 1.4), even when 8L and 8H sites were removed from the PCA. This suggests that farmer soil fertility distinctions were more related to crop-available P than to soil C or N content.

Principal component 3 (PC3) loaded soil % clay and % sand, showing that soil texture was a parameter distinct from management for this set of fields that was most related to soil parent material and/or erosion of original soil horizons.

Table 1.2. Loadings of site soil variables on orthogonal rotated factors using varimax rotation of first four principal components (PCs) from 50 experimental blocks accounting for 87% of the variation among measured site variables. Transformations of variables to correct non-normality are shown. For clarity, loadings < 0.25 have been left blank, and those > 0.50 have been placed in bold text.

Rotated PC factor (PC)	PC1	PC2	PC3	PC4
Percent variation explained	**28.4**	**27.2**	**17.8**	**13.5**
Soil pH	**-0.85**			0.32
Ln(Olsen-P_{inorg})	**0.83**	0.37		
Bicarb-$P_{org.}$	0.42	**0.83**		
Ln(NaOH-$P_{inorg.}$)	**0.91**			
Ln(NaOH-$P_{org.}$)	**0.70**	**0.56**		
Ln(DHCl-$P_{inorg.}$)				**0.87**
Soil tot %C	0.28	**0.92**		
Soil tot %N		**0.93**		
Ln(% soil clay)			**0.93**	
soil % sand			**-0.90**	
Avg site growing season air temp.	-0.31		0.39	**0.67**

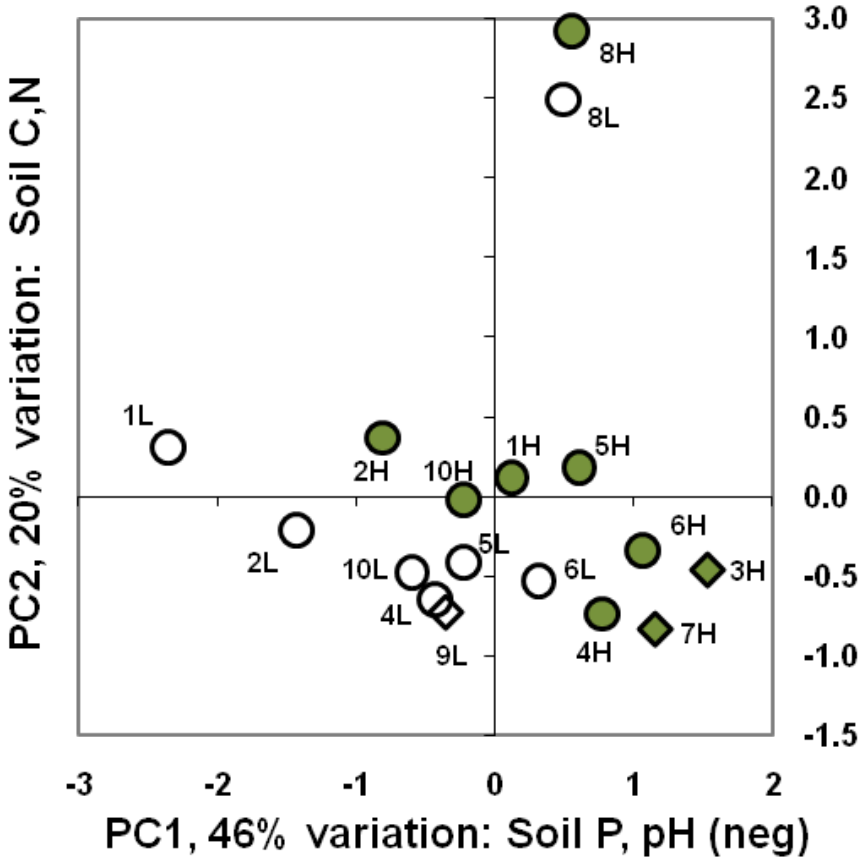


Figure 1.4. Biplot of PC 2 vs. PC 1 showing differences among sites relevant to farmer fertility designations. Farmer designated high fertility are dark symbols and low fertility are open symbols. Diamond symbols are sites from 2005-06 while circles are sites from 2006-07.

Meanwhile principal component 4 (PC4) had the strongest loading from the DHCl-P_i pool linked to soil calcium phosphates (Ca-Ps), followed by site temperature (an elevation proxy). Soil pH loaded weakly and positively on PC4, consistent with higher soil pH at lower elevations and accumulation of Ca-P in soil of pH 7 or above (e.g. sites 1L, 1H, 4L). In addition to these probable climate effects on Ca-P, site soil data also indicated links between Ca-P and soil fertility management. Using the data from all 17 sites including 1L, 1H, 4L, correlation between Olsen P_i and DHCl-P_i was R=0.17 (ns). Ignoring low elevation, high Ca-P sites, this correlation is much stronger (R=0.44, p=0.004) due to low-pH, P-fertile fields with appreciable measures of dilute HCl-P_i.

Added P impacts on biomass, N and P assimilation, and N fixation

The impact of P addition on legume productivity was positive on average but varied among sites (Table 1.3). Averaged across all sites, total legume N uptake in shoots (N_{total}) and showed a 28% increase with RP addition and a 43% increase with TSP addition, ignoring soil covariate interaction effects ($p<0.05$). Site-specific data in Table 1.3 includes species means, and species effects and interactions are included in statistical models, but species differences are not emphasized in this paper, since P addition x species interaction was not a significant term in any of the mixed statistical models we used. Differences between vetch and lupine at ambient fertility and are reported elsewhere compared to two additional legume species (Vanek, Chapter 2). In spite of these positive overall effects, results only partially confirmed that RP and TSP would have maximum impact at low P fertility. Table 1.3 shows field by field impacts on legume N_{total} with three possible outcomes for P addition: no significant impact of either form of applied P; fields where only TSP increased N_{total}, and fields where both forms of P addition increased N_{total}. Seven of nine fields that responded

Table 1.3. Site-level results for total N uptake in roots and shoots, (fixed +soil-derived N), for vetch and lupine. Percent increase in N uptake and statistical significance of increase with RP and TSP addition is shown at right (significance of contrast comparing RP or TSP to the 0P control: *= p<0.05 level; **= p<0.01 level). L and H show sites designated as low and high fertility by farmers.

Site	Site Elevation (m.a.s.l)	Season rainfall (mm)	Vetch total N uptake ($kg \cdot ha^{-1}$)	Lupine total N uptake ($kg \cdot ha^{-1}$)	Percent increase with RP addition	Significance of RP addition	Percent increase with TSP addition	Significance of TSP addition
				2005-06 sites				
3H (05)	3100	800	210	143	2		76	**
7H (05)	3600	980	200	287	0		29	
9L (05)	3950	770	57	69	76	*	37	
				2006-07 sites				
1L	2700	550	68	no biomass	-5		58	*
1H	2730	550	34	no biomass	26		7	
2L	3010	630	81	25	263	*	347	*
2H	3000	630	218	54	-20		11	
4L	3100	700	83	19	58		49	
4H	3150	700	46	109	41		179	*
5L	3500	620	43	152	22		115	*
5H	3500	620	29	138	-4		71	*
6L	3550	650	76	207	29		99	*
6H	3600	650	grazed	247	-3		20	
10L	3950	560	204	213	96	**	154	**
10H	3950	560	177	109	76	**	104	**
				2006-07 sites with high organic matter soils				
8H	3700	620	194	194	6		not applied	
8L	3750	650	discarded[1]	163	101	*	31	

[1] Mean N uptake aboveground was <5 $kg \cdot ha^{-1}$ and root biomass was not assessed.

to some form of P addition were L fields, suggesting that P fertility played a role in defining responsive sites (Table 1.3). However legumes in half of L fields did not respond to RP addition, indicating that the link between low P fertility and P addition impacts was weakest for RP addition. Also, TSP increased legume N_{total} at four H sites, suggesting P-limitation even at these sites. In a mixed model for N_{total}, farmer classification of sites was not significant in interaction with P addition ($p=0.25$) and thus did not predict positive legume response to P addition.

Because soil P fertility alone did not explain differences in the impact of added P, we used soil properties to interpret differences in P response among fields. Before presenting PCs one through four from the PCA as covariates, 11 soil variables from the PCA were used alone as covariates that show direct positive or negative effects on legume fixed N (Ndfa), or interaction effects with P addition (Table 1.4). P fertilization had larger effects on legume Ndfa in soils with lower clay content and dilute HCl-P_i in soils, indicating that these two soil properties explain field-level variation in primary legume responses like N fixation and biomass.

Using covariates extracted from the PCA (PCs 1-4) confirmed the importance of soil texture and dilute HCl-P_i (PC3 and PC4) in differential effects of P fertilization, and showed that biomass was the main source of response to P addition. Attributes related to biomass – N_{total}, Ndfa, and total P assimilation – responded similarly to P addition with larger increases in soils with light textures (PC3) and low dilute HCl=P_i/site temperature (PC4; Tables 1.5, 1.6, Figs. 1.5, 1.6). Overall productivity rather than proportion N fixed or P content was thus the main source of response to P addition. Indeed, Ndfa was highly correlated to total biomass C ($R=0.93$, $p<0.0001$) as well as to P uptake ($R=0.90$, $p<0.0001$). These results supported the hypothesis that

Table 1.4. Significance of soil characteristics used as covariates in a mixed model analyzing effects of P addition and species on total Ndfa. Soil parameters were log-transformed where deviations from normality occurred.

		Significance of variable as a covariate[1]		
Soil parameter covariate	**Parameter estimate**	**Main effect**	**Interaction with P addition**	**Interaction with species**
Soil pH	-0.56	*		***
ln(Olsen P)				**
Bicarb-$P_{org.}$				*
ln(NaOH-$P_{inorg.}$)				***
ln(NaOH-$P_{org.}$)				***
ln(DHCl – $P_{inorg.}$)			*	
Soil tot %C	+0.51	*		
Soil tot %N		+		
ln(% soil clay)			*	***
soil % sand				***
Avg. site growing season air temp.		*	+	***

[1] Significance of variable (e.g. soil pH) used as covariates in a statistical model describing total NDFA for shoots and roots in year two; + = 0.10 level; * = 0.05 level; ** = 0.01 level; *** = 0.001 level. For interaction effects, each covariate was tested alone in interaction with the factor of interest (P treatment or species) while retaining in the model significant main effects and interactions with the other factor.

Table 1.5. Mixed-Model ANOVA results for Shoot N, amount N derived from atmosphere (Ndfa), amount N derived from soil (Ndfs), Percent Ndfa, and total P uptake in roots and shoots. Species by PCA factor interactions are not shown but are included in model when significant.

	Total N uptake, two years' data, kg·ha^{-1}		NDFA amount, year 2 only, kg·ha^{-1}		NDFS amount, year 2 only, kg·ha^{-1}		% NDFA, year 2 only		total P uptake in shoots and roots, year 2 only, kg·ha^{-1}	
random effects	Random effect variance components									
site % variance	55		61		67		44		69	
block within site % variance	0		1		7		5		2	
% residual in random effects	45		38		26		51		29	
fixed effects	F ratio	p value	F ratio	p value	F ratio	p value	F ratio	p value	F ratio	p value
	Treatment factor fixed effects									
Species	2.1	ns	11.3	**0.001**	16.5	**0.01**	60.9	**<.0001**	4.92	**0.03**
P addition	36.9	**<.0001**	29.4	**<.0001**	25.4	**<.0001**	3.8	**0.02**	47.9	**<.0001**
	PCA-derived covariates, main effects									
PC 1: Soil inorganic P and pH	5.1	**0.03**	1.9	0.18	2.4	0.15	**ns**	**ns**	3.0	0.07
PC 2: Soil organic matter	1.5	ns	1.1	**ns**	3.3	*0.07*	**ns**	**ns**	3.4	**0.04**
PC 3: Soil texture	0.7	**ns**	0.0	**ns**	0.0	**ns**	**ns**	**ns**	0.0	**ns**
PC 4: Soil DHCl- P_i, site temp.	1.3	**ns**	0.9	**ns**	0.2	**ns**	**ns**	**ns**	0.0	**ns**
	PCA-derived covariates, interaction with P addition									
P addition x PC 1	ns	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	2.4	0.12
P addition x PC 2	ns	**ns**	2.7	0.07	2.4	*0.09*	**ns**	**ns**	ns	**ns**
P addition x PC 3	3.4	**0.04**	3.6	**0.03**	3.2	**0.046**	**ns**	**ns**	3.5	**0.02**
P addition x PC 4	4.0	**0.02**	7.5	**0.001**	4.9	**0.006**	**ns**	**ns**	8.7	**0.000**

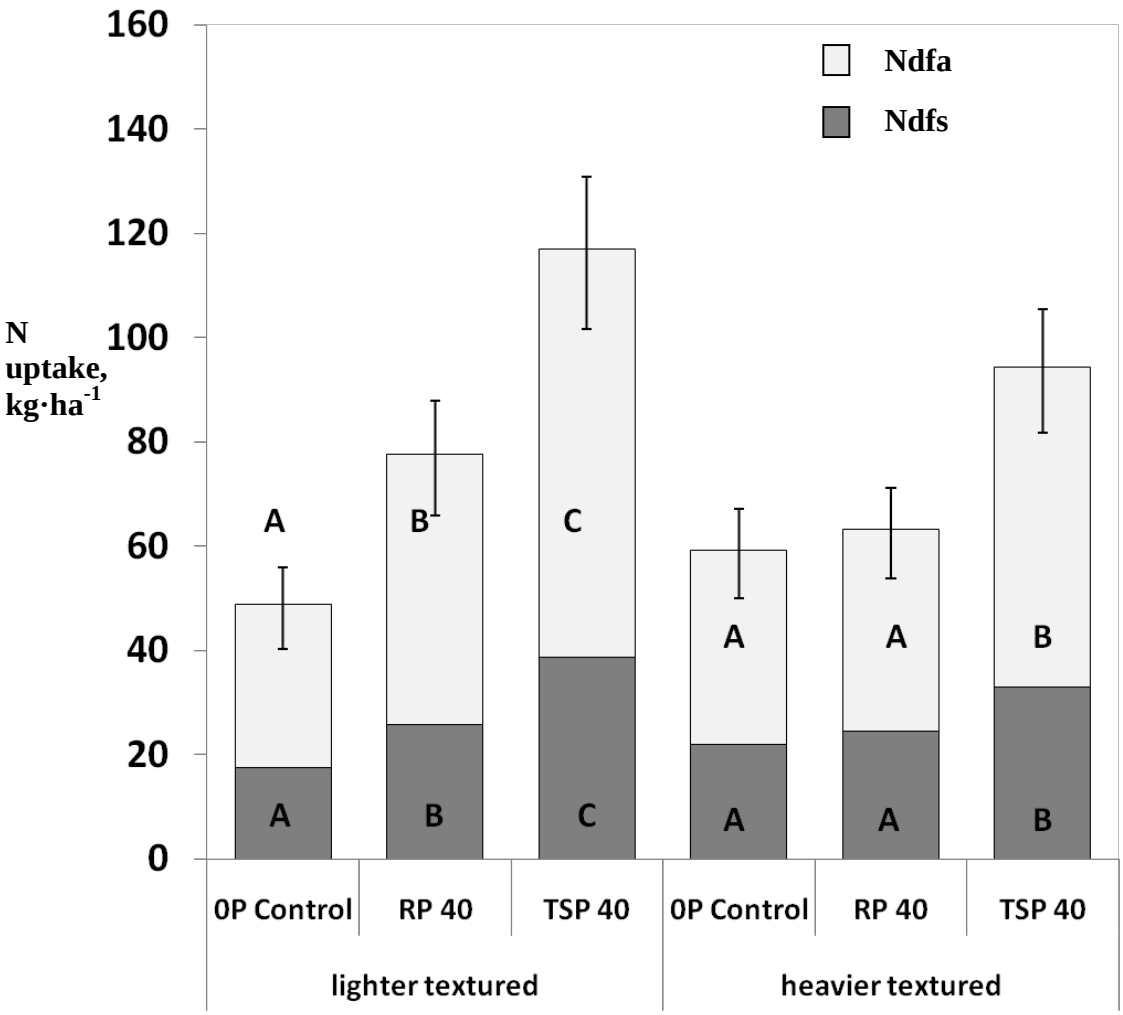


Figure 1.5: **Fixed N and N from soil for different P addition treatments along the soil gradient for soil texture (principal component 3)**. N from soil (Ndfs) is shown as dark bars and fixed N (Ndfa) as light bars. Bars represent the total for shoots, roots, and nodules. Means were evaluated at the -1 and +1 value of the standardized PC3 for texture. Different letters denote significantly different P treatment means for the N uptake fraction within the given value of PC3.

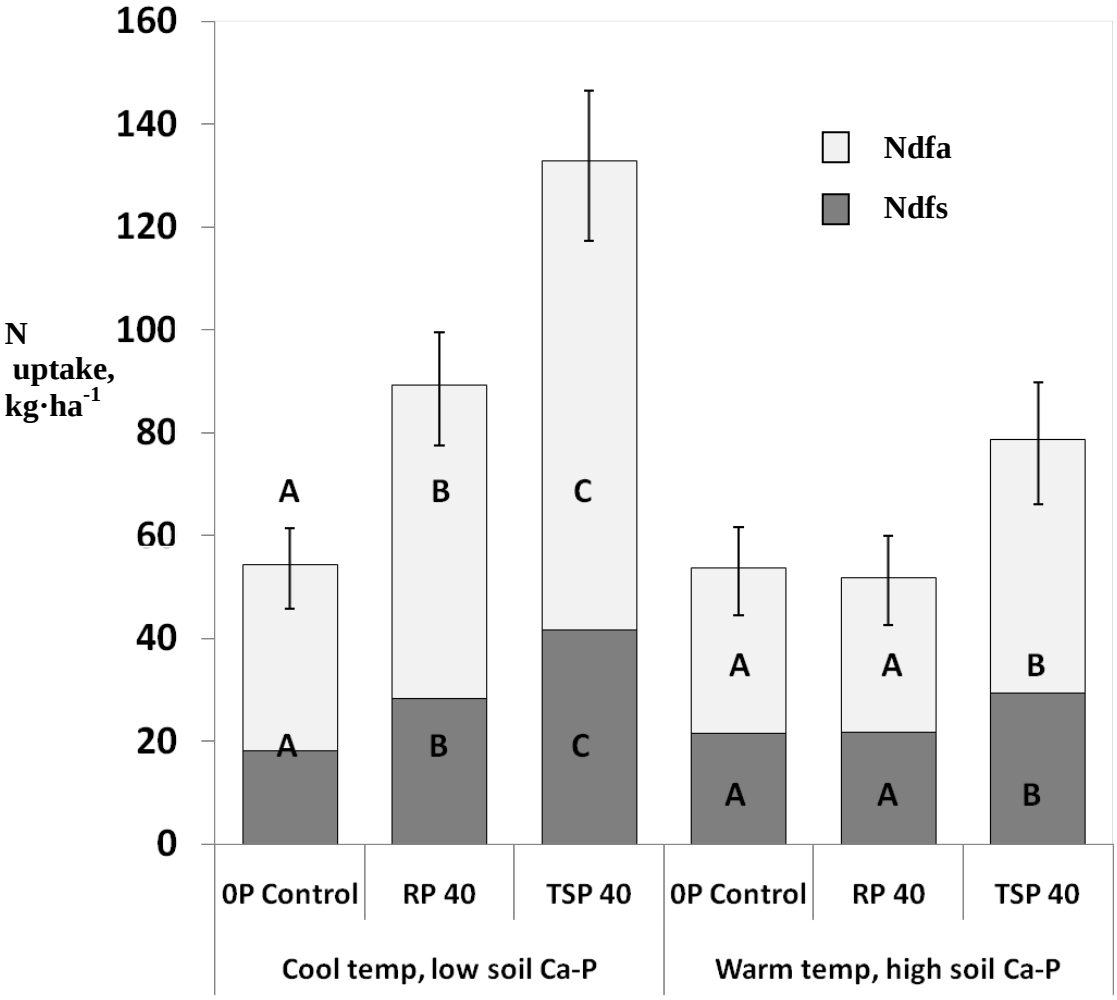


Figure 1.6: **Fixed N and N from soil for different P addition treatments along the soil gradient for soil dilute HCl-P_i / site temperature** (principal component 4). N from soil (Ndfs) is shown as dark bars and fixed N (Ndfa) as light bars. Total shown is for shoots, roots, and nodules. Means were evaluated at the -1 and +1 value of PC4 for dilute HCl-P_i and temperature. Different letters denote significantly different P treatment means for the N uptake fraction within the given value of PC4.

added P expanded total biomass and associated nutrient stocks by increases in Ndfa.

Where unconstrained by high clay contents, Ndfa increased by 65% with RP and over 100% with TSP, while increases in Ndfa occurred only with TSP addition in high-clay soils (Fig. 1.5). Increases in Ndfa and Ndfs in light-textured soils occurred with increases in total P uptake that were 70% higher for RP addition and almost three-fold for TSP addition (Table 1.6). Total belowground C (Table 1.6) and fine root C (not shown) also increased in light-textured soils. Increases in belowground C supported our hypothesis that P addition would lead to favorable impacts on both shoot and root residues. Root residues would be important for soil impacts of vetch and lupine if crop shoots were harvested.

P addition also had larger effects on Ndfa, Ndfs, and P assimilation in fields with lower soil dilute HCl-P_i and site temperature (Fig. 1.6). This interaction effect could have resulted from factors related either to the presence of calcium phosphates linked to the dilute HCl-P_i fraction, or to drought stress that hampered productivity and P uptake at warmer, lower sites. We tested these two factors separately, inserting ln(DHCl-P_i) and site temperature as separate covariates substituting for PC4 in the mixed model for Ndfa. Ln(DHCl-P_i) was significant at $p=0.02$ in interaction with P addition, while temperature was not significant ($p=0.53$). Sites with cool temperatures and high soil dilute HCl-P_i (e.g. fields 8H and 6H, Fig. 1.2d) were unresponsive to P addition (Table 1.3), confirming that DHCl-P_i was a stronger factor than site temperature in altering P response.

Similar to total P assimilation, P addition increased amounts of P in nodules, roots, and shoots to a greater degree at sites with lower soil dilute HCl-P_i (Table 1.6).

Table 1.6. Legume responses reflecting interaction between P addition and soil texture (PC3, top), elevation / soil Ca-P (PC4, middle), and inorganic P fertility (PC1, bottom)

Means for three left data columns are evaluated for each standardized PC equal to -1, i.e. lower clay content, temperature/Ca-P pools, and inorganic P fertility respectively. Means at right are evaluated for each PC= +1, i.e higher clay content, temperature/Ca-P, etc. Within each row and set of three columns, letters denote significant differences with $p<0.05$.

PC 3: soil texture	**low clay content (*PC3= -1*)**			**high clay content (*PC3= +1*)**		
	0P control	**RP 40**	**TSP 40**	**0P control**	**RP 40**	**TSP 40**
Aboveground P stock ($kg \cdot ha^{-1}$)	**1.8**	**3.3**	**6.3**	**2.5**	**2.9**	**5.1**
	a	b	c	a	a	b
Belowground biomass C[1] ($kg \cdot ha^{-1}$)	**431**	**535**	**698**	**444**	**438**	**541**
	a	b	c	a	a	b
PC 4: Ca-P pools , site temperature	**Cool sites, low DHCl-P_i (*PC2= -1*)**			**Warm sites, high DHCl-P_i (*PC2= +1*)**		
	0P control	**RP 40**	**TSP 40**	**0P control**	**RP 40**	**TSP 40**
Aboveground P stock ($kg \cdot ha^{-1}$)	**1.9**	**3.8**	**6.8**	**2.4**	**2.4**	**4.5**
	a	b	c	a	a	b
Belowground P in biomass (roots+taproots+nodules, $kg \cdot ha^{-1}$)	**1.5**	**2.0**	**2.8**	**1.4**	**1.8**	**2.6**
	a	b	c	a	a	b
Nodule P ($kg \cdot ha^{-1}$)	**0.09**	**0.15**	**0.25**	**0.11**	**0.12**	**0.20**
	a	b	c	a	a	b
Belowground biomass C ($kg \cdot ha^{-1}$)	**423**	**554**	**751**	**455**	**409**	**474**
	a	b	c	ns	ns	ns
Fine root biomass C:P ratio	**340**	**256**	**224**	**272**	**246**	**224**
	a	b	b	a	ab	b
PC 1: soil inorganic P	**Low inorganic P pools (*PC1= -1*)**			**High inorganic P pools (*PC1= +1*)**		
	0P control	**RP 40**	**TSP 40**	**0P control**	**RP 40**	**TSP 40**
Nodule P ($kg \cdot ha^{-1}$)[2]	**0.04**	**0.09**	**0.15**	**0.25**	**0.21**	**0.37**
	a	b	c	a	a	b
Shoot biomass C:P ratio	**281**	**245**	**205**	**208**	**224**	**213**
	a	b	c	ns	ns	ns
Fine root biomass C:P ratio	**343**	**255**	**223**	**258**	**246**	**227**
	a	b	b	ns	ns	ns
Ndfs per P in whole biomass[3]	**7.7**	**6.0**	**5.2**	**4.5**	**5.2**	**4.2**
	a	b	b	ns	ns	ns

[1] Total of C in fine roots, tap roots, and nodules

[2] Main effect of PC1 significant, p=0.01.

[3] Main effect of PC1 for Ndfs weakly significant, p=0.051. Ndfa per P was not significantly different among P addition treatments, nor were any covariates significant in interaction with P addition.

Proportional increases were however larger in the case of shoot and nodule P pools relative to root P pools (260% and 180% increase in shoot and nodule P from 0P to TSP vs. 90% for total belowground biomass). This suggested preferential allocation of P to shoots and N fixation in nodules over root biomass, which supported hypotheses based on plant functional equilibrium. Nodule P was also increased almost three-fold by a main effect of soil P fertility (PC1; Table 1.6). P addition also increased nodule P to a greater degree at sites with low P fertility than at P-fertile sites. These nodule responses to P addition and ambient P fertility reinforced the strong role played by P in N fixation of legumes to support the range of primary-biomass driven responses to P addition.

Percent soil cover, root:shoot ratios, and proportion N fixed

Percent soil cover by legumes at midseason differed significantly among all three levels of P addition, an effect that did not differ with soil properties (Fig. 1.7). Cover was increased by almost 30% in the case of RP addition and by over half with TSP addition. Large proportional increases, and absolute values greater than 30% are encouraging, since 30% is a threshold for soil cover that is thought to reduce soil erosion (Duran Zuazo and Rodriguez Pleguezuelo, 2008). Added P of either type would thus shorten the period that soil is unprotected after crop seeding.

In agreement with plant functional equilibrium theory, P additions increased shoot:root ratio of legumes (Table 1.7), with no differential impacts based on soil properties. As we had hypothesized, increased shoot:root ratios were accompanied by increases in both shoots and root biomass under P addition (Figs. 1.5 and 1.6, Table 1.6). Amounts of shoot and root residues for subsequent soil impacts would thus both be increased.

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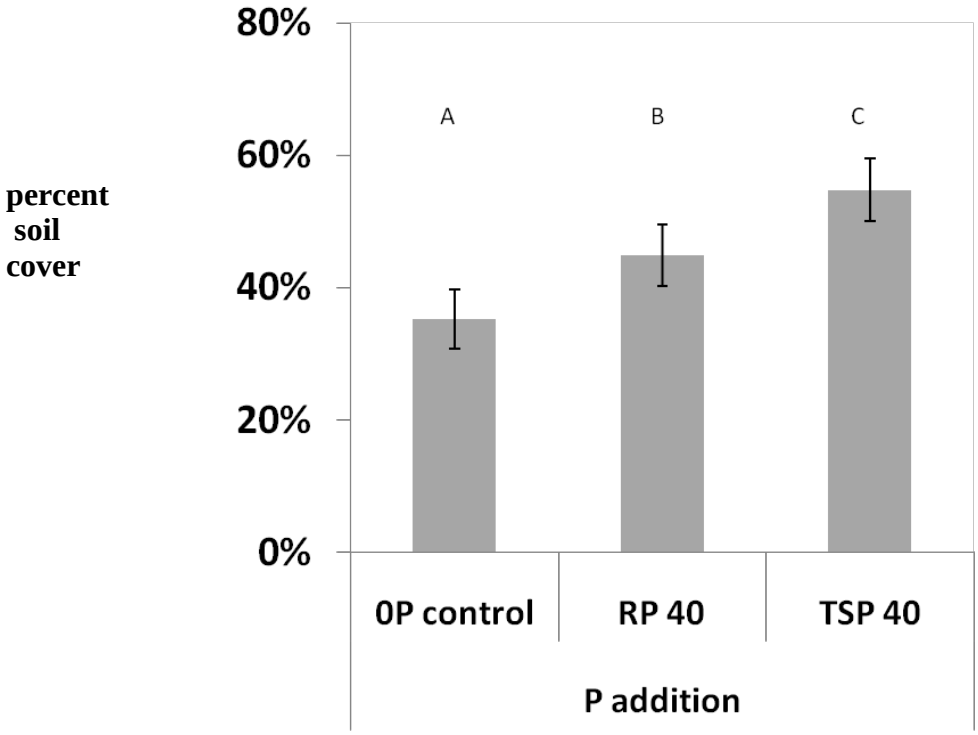

Figure 1.7. Visually estimated % cover of soil by legumes 75 days post-seeding of crops. Interactions of P addition with PCA-derived factors (PC 1 through 4) were not significant

Table 1.7. %Ndfa, Shoot:root biomass C ratio, and C:N of shoot and fine root biomass. These response variables did not interact significantly with rotated factors describing site parameters (PC1 through 4)

	P addition		
	0P control	**RP 40**	**TSP 40**
% N derived from atmosphere	59.1 a	60.2 ab	63.7 b
Shoot: root C ratio	1.3 a	1.6 b	2.0 c
Shoot C:N ratio	16.5	16.4	16.1
	----------ns----------		
Fine root C:N ratio[4]	20.0 a	19.5 ab	19.0 b

[4] Main effect of PC2, C:N ratio, significant at P=0.001, parameter = -1.14 decline in root C:N ratio per unit standard deviation of the covariate

Added TSP increased the proportion N fixed (%Ndfa) only modestly compared to the control, from 59 to 64% (Table 1.7). This small increase in %Ndfa with TSP was dwarfed by large increases in productivity, P uptake, and total N fixed with P addition, reinforcing that productivity and total nutrient assimilation were dominant responses to P addition, rather than variation in one particular process like N fixation.

The relation of soil pH to N fixation

Soil pH was important as a significant main effect and had differential impacts with P addition on species (Table 1.4). It is important to explore the positive effect that low soil pH had on N fixation, given that neutral rather than low soil pH is generally thought to favor BNF in temperate species (Jaarsveld et al., 2002; Lapinskas and Piaulokaite-Motuziene, 2006). In our experiment low soil pH was associated with high P fertility in sites at middle to high elevation so that they loaded together and with an opposite sign on PC1. This was likely a real parallel gradient based on hydric balance of soils in this mountain agroecosystem, rather than an accidental confounding effect of site selection. At these low pH, high fertility sites, N fixation was generally high so that the main effect of higher pH was negative (Table 1.4). Also, vetch fixed more N in higher pH environments while lupine fixed substantial N even at low soil pH in these high elevation sites, driving a negative main effect of soil pH combined with a significant interaction of soil pH with species (Table 1.4).

Secondary effects of P addition: residue C:P and C:N quality

C:P ratios for shoot and root residues were narrowed by P fertilization at low soil P fertility (PC1) and showed no change at high soil P fertility (Table 1.6). Narrower C:P ratios for roots occurred with increases in belowground biomass with TSP addition (data not shown; Table 1.6 shows similar increases in root C at low values of PC4). P addition thus increased both root biomass and root residue quality at sites with low measures of soil fertility. This suggests that even with harvest of

shoots by farmers, P addition would increase quantity and quality of root residues and improve soil P fertility impacts via residue decomposition.

These changes in residue quality might seem to refute our hypothesis that P fertilization would not alter residue quality due to parallel increases in C, N, and P with biomass. However, rather than exhibiting 'luxury uptake' of P as other nutrients become limiting, narrowed C:P ratios were consistent with alleviation of a plant nutrient stress (Table 1.6). For the case of roots, at low levels of available P (Olsen-P_i or DHCl-P_i) legumes were more P-efficient in growing roots, with less P per unit root biomass and resulting higher C:P. This is seen in the middle and bottom of Table 1.6, where root C:P is reduced from ~340 to ~265 by increases in ambient Ca-P (PC4) and other inorganic P pools(PC1). P fertilization alleviated this stress: it narrowed root C:P ratio while increasing biomass, rather than being associated with growth limitation. Aside from P stress alleviation, other results for C:P show no significant changes (Table 1.6), supporting our hypothesis that C, N, and P scaled together with biomass and that legumes never reached a threshold of 'luxury uptake'.

Results for C:N residue quality of legumes confirmed our hypothesis that no change would result as both C and N stocks were increased by P addition. Shoot tissue C:N was not significantly different across any of the P treatments, and declined only modestly from 20 to 19 in the case of root biomass (Table 1.7), reinforcing that impacts of crop residues would likely result more from changes in biomass than C:N ratio.

Mycorrhizal colonization

Mycorrhizal (AM) colonization was reduced with soluble P addition at sites with low soil organic matter (SOM) but not at sites with higher SOM and higher levels of N availability (Table 1.8). These results were consistent with the trade balance model of plant-AM relations. Mycorrhizal colonization of Andean lupine was near

zero, consistent with *Lupinus* generally being non-mycorrhizal. For vetch AM colonization was reduced at sites with higher P fertility (PC1), as predicted by the trade balance model. Meanwhile, colonization was reduced by TSP application at sites with low levels of SOM (PC2), but not sites with high SOM levels (Table 1.8). No reductions in AM colonization were seen with RP application. These P fertilization results were also interpretable using the trade balance model. Higher SOM (PC2) was associated with higher levels of N uptake from soils by both legumes and the oat reference crop ($R=0.40$, $p=0.02$ between PC2 and Oat N uptake). Thus differential response of colonization to P addition may have resulted from more available soil N at high-SOM sites, where even with P addition, soil N:P ratios were not altered sufficiently to prompt reductions in colonization. To further support this conclusion, we tested the interaction of P addition with one measure of soil N:P, the ratio of total soil N (from PC2) to two inorganic P pools (from PC1) or soil $N:P_{inorg}$. The P treatment by $N:P_{inorg}$ interaction was significant ($p=0.02$) and showed the same pattern PC2 (Table 1.8), consistent with the idea that variations in both N and P fertility may have altered the response of AM colonization to TSP addition.

Discussion

Farmer fertility designations and soil gradients

Farmer H/L fertility designations were predictive of dramatic differences in available P (PC1), soil C, site slope, and fields' distance from households. PC1 from the PCA linked farmer designations to a main axis of variation in soil P fertility. Differences between sloped, C- and P- impoverished outfields and high fertility infields are consistent with our work on nutrient balances, where larger applications of manure P by farmers in near fields and erosion P losses on steeper outfields created

Table 1.8: Mixed-effect ANOVA results for mycorrhizal colonization of vetch using PC covariates and soil total N:P_{inorg} ratio. Percent mycorrhizal colonization of vetch roots under different P addition treatments for 8 sites spanning gradients in soil texture, inorganic P, and total soil C and N.

Significant fixed effects and interactions	F ratio	p-value
P addition	**7.4**	0.002**
PC1, soil P fertility (parameter: -0.11)	**9.5**	0.007**
PC2, soil organic matter	0.2	ns
Average site temperature (from PC4, parameter = -0.025)	**7.3**	0.054^{+}
P addition x PC 2: Soil C,N, Organic P pools	**5.9**	0.005**

Mean separation for % colonization at two levels of PC2 (soil organic matter) and two levels of soil N:P_{inorg}

	P addition treatment		
	0P	**RP40**	**TSP40**
PC2, soil C, N = - 0.5	58 a	55 a	45 b
PC2, soil C, N = + 0.5	49 ns	46 ns	51 ns
Ln(soil N:P_{inorg})[5] = 3.00	53 a	48 ab	39 b
Ln(soil N:P_{inorg}) = 3.75	59 ns	58 ns	53 ns

[5] Covariate was log-transformed to satisfy conditions of normality. For use in modeling and presentation, ratio was also divided by 100 for ease of presentation. Ln (Soil N:P_{inorg} /100) was significant as a main effect with +0.12 increase in arcsine-square root transformed percent colonization per unit increase in the log-transformed ratio.

more negative P balances for infields (Vanek, Chapter 3). This P fertility gradient underscored the need to reverse degradation by raising P-fertility status of outfields.

In contrast to PC1, which was largely management-driven, PC2 (soil C, N, organic P) reflected SOM accumulation typical of high elevation Andean environments (Egashira et al., 1997). PC2 probably also reflects management in a longer timeframe than PC1, either maintenance of SOM via nutrient inputs or degradation and SOM loss via tillage and erosion.

PC4 (Dilute HCl-P_i, temperature) combined temperature, a site factor related to elevation, with the dilute HCl-P_i (DHCl-P_i) pool related to both site factors and management. In most high-elevation fields, DHCl-P_i probably resulted from fertility additions, but high DHCl-P_i at low elevation was likely linked to accumulation of calcium phosphates (Ca-P) in higher pH and drier soils, similar to high Ca-P found in aridisols by Lajtha and Schlesinger (1988) and in agreement with positive correlation between pH and DHCl-P_i found by Herlihy et al. (2007). DHCl-P_i pools at low-elevation sites may have also reflected primary P in parent material. The experiment was located within 50 km of known RP deposits (Luisaga and Camacho, c. 1985) and the 4L and 1L sites with high DHCl-P were sloped, low soil C sites with eroded A horizons and potentially strong contributions of bedrock parent material. Of relevance to basic understanding of the effectiveness of RP is that Ca-P from divergent sources (management, hydric balance and pH, parent material) seemed to have the same interaction with P addition in diminishing the impact of RP.

Legume response to P addition

Impacts of applied P were substantial, especially on biomass and other primary plant responses that contribute directly to improvement of soil nutrient cycling. Legumes responded to P addition in accordance with predictions of nutrient limitation and functional equilibrium theories. Soil limitations to P assimilation are discussed

further on, but it is clear that once inside the plant, P was used by legumes in a coordinated way that increased N and C assimilation, soil cover, shoot:root ratio, and root biomass. These primary impacts on legumes were encouraging for the use of additional P and legumes in these cropping systems.

C:N and C:P of legume residues, a secondary attribute for nutrient cycling, did not vary greatly under P addition, which we argue resulted from parallel increases in C, N, and P that support the microeconomic analogy for nutrient limitation put forth by Bloom et al. (1985). An exception to this was the wider C:P ratios at low available soil P, which represented greater P use efficiency under P stress rather than a refutation of the microeconomic paradigm (Table 1.6).

In low fertility fields, where rehabilitation of nutrient cycling is most necessary, our results suggest that P effects on biomass and residue quality could work together to foster virtuous cycles towards greater soil fertility. The large impacts on biomass C, Ndfa, and residue P stocks from P addition would be augmented by narrower C:P ratios and greater likelihood of P release with decomposition to successive crops. Changes in P release from legume residues due to C:P ratio has been demonstrated by other research. For example Abarchi et al. (2009) showed that for a range of C:P ratios similar to those we found (C:P ratios estimated based on their data as between 150 and 300), C:P ratio of legume residues predicted differences in P release under incubation. Thus, although increases in total residue stocks would be the dominant impact of P addition, secondary impacts like those from residue quality at P-infertile sites might complement these positive effects on nutrient cycling.

Smaller increases in %Ndfa with P fertilization than for amount Ndfa agree with other results on P addition to legumes (Somado et al., 2006). These results also suggest that TSP addition altered tradeoffs for vetch between BNF and AM symbioses. TSP additions increased not only %Ndfa but also nodule C and P

allocation, while causing reductions in vetch AM colonization (for low-SOM sites). Co-occurring increases in %NDFA with reductions in AM colonization suggest that supplying legumes with soluble P with lower C costs for acquisition freed greater amounts of C and P for allocation to N fixation. These tradeoffs were consistent with the microeconomic paradigm of plant nutrition (Bloom et al., 1985).

The increased soil cover we observed with applied P is encouraging for promoting positive trends in soil regeneration because it would strengthen trends towards improved soil fertility by retaining applied P in farmers' fields, a phenomenon explored for bean genotypes by Henry et al. (2010). Increases in soil cover by legumes with added RP or TSP at midseason did not vary in interaction with soil factors, and occurred across the gradients in soil type and P fertility we characterized. This contrasted with the differential effects with soil type of P addition on legume productivity when biomass was sampled a few months later. The comparison in these two results may indicate that soil sorption and other constraints posed by soil factors to P addition occurred incrementally as the season went on, while an initial amount of applied RP or TSP was immediately available to legumes.

Soil constraints to added P availability for legume responses

All but one site responsive to RP addition had low P fertility (PC1), suggesting that low soil P fertility was conducive to impacts of applied P. However, Ca-P status (DHCl-P_i) was a better predictor than overall P fertility of legume response to RP addition, in agreement with Khawsaneh and Doll's ideas that Ca-P status, texture, and pH are factors regulating dissolution of RP (1978). Given these authors' work, we would expect that under conditions with high Ca-P, RP dissolution via acidification in the rhizosphere by legumes would indeed be hampered.

Clays and associated metal oxides, including silicate clays likely present in these soils, have been characterized as a sorption sink for applied P (Demolon et al.,

1953; Herlihy and McGrath, 2007; Khasawneh and Doll, 1978; Mamo and Wortmann, 2009) Although sorption by clays can hasten dissolution of RP by lowering concentrations of dissolution products from apatite (Smyth and Sanchez, 1982), clay sorption can also obstruct uptake of solubilized P, as suggested by reduced impacts of both TSP and RP on BNF in heavy textured soils in our experiment. This conclusion is reinforced in our results by low recovery of applied TSP in high-clay soils: only 3.5 kg or roughly 9% of the 40 $kg \cdot ha^{-1}$ TSP applied was recovered in legume biomass in heavy-textured soils, compared to 14% for light-textured soils (Table 1.6).

Soil pH also likely played a role in determining the effectiveness of P additions to legumes, although our experiment did not detect it as a dominant constraint because only two sites had truly alkaline pH. Interestingly, four of five responsive sites to RP had a pH of 6 or greater (but low clay content and Ca-P status), suggesting that RP impacts can occur even under mildly acidic soil conditions when soil pH buffering, P sorption by clays, and Ca-P concentrations are low enough to allow dissolution of RP through inherent soil pH as well as rhizosphere acidification by legumes. At the two lowest elevation sites (1L, 1H) with alkaline pH and high levels of dilute HCl-P_i, RP addition was not effective, consistent with this explanation. These soil pH conclusions are in agreement with Perez et al.'s (2007) tests of RP impacts, in which RP response in a grass and legume occurred at both pH 4.9 and 5.8.

In summary, our work supports the idea that an intersection of low soil P status, low Ca-P status, light-textured soils, and soil pH below 7 are necessary for RP impact on legume function in smallholder farming systems. Management plays a role in these factors as regards soil P fertility, but a farmer soil fertility typology reflecting inorganic P pools did not by itself discriminate limitation of BNF and legume biomass by RP. Soil texture and calcium phosphates from a variety of sources were a stronger constraint than management and soil fertility. Conversely, P impacts on legumes did

sometimes occur in farmer-designated high fertility fields when soil texture and low Ca-P pools allowed. Knowing about such soil constraints to fertility interventions is important more generally to maximize the impact of innovations in agroecosystems.

Mycorrhizal symbioses and the trade balance model

Our results on AM colonization extended the trade balance model into the realm of smallholder agro-ecosystems, and suggest that more research on legume-AM symbioses in smallholder agriculture could be beneficial. The trade balance model was drawn from pot studies and work on natural ecosystems such as grasslands (Johnson et al., 2003) Fertilization impacts on both colonization and on AM community composition have been investigated (Asmah, 1995; Johnson, 1993; Nijjer et al.), but little work has been done testing these theories in low-input agricultural systems where we would expect that AM are playing a strong mediating role between mycorrhizal legumes and soil nutrient pools.

We argued that P fertilization impacts on AM colonization resulted from changes in soil N:P brought about by soluble P addition and ambient levels of N linked to SOM levels (PC2), in accordance with the trade balance model. The explanation we propose is that under high levels of soil $N:P_{inorg}$, a situation described by Johnson (2010) as fostering the greatest degree of plant-AM mutualism, additional soluble P and consequent narrowing of the $N:P_{inorg}$ ratio was insufficient to reduce colonization rates. At lower levels of ambient soil $N:P_{inorg}$ linked to lower SOM levels (PC2), P addition altered the $N:P_{inorg}$ ratio sufficiently to reduce colonization rates. There are alternate explanations for this result that we were not able to test. One is that higher-SOM sites had different and more parasitic AM species, so that legumes provided soluble P had similar colonization rates as unfertilized plants. . This interpretation was suggested by Corkidi et al. (2002), who reported that fertilized soils promoted less mutualistic AM fungi compared to those in unfertilized soils. More

research would be needed to extend and test the ideas of the trade balance model, ideally in smallholder agroecosystems with practices not heavily reliant on soluble nutrients and reliance on AM symbionts for P nutrition.

Reductions in AM colonization with TSP addition at low-SOM sites suggest that using fertilizer only to boost legume BNF, rather than RP or an organic source of P that supports greater rates of colonization, could impoverish AM symbioses of mycorrhizal legumes, or foster more parasitic plant-AM relations. Soluble P application had large beneficial impacts on BNF, but considering the role of AM symbioses argues for modest amounts of P fertilizer addition and/or use of relatively insoluble organic and RP forms of fertility.

Management implications

Agronomic impacts of P addition reported here agree with research showing P-limitation of BNF in smallholder agriculture and rapid impact of RP and soluble P (Perez et al., 2007; Somado et al., 2006). Under the suitable soil conditions we document, soluble P and RP additions could radically increase legume impacts for production and soil regeneration in smallholder agroecosystems. Augmented Ndfa, soil coverage, and nutrient stocks with narrower C:P ratios at P-infertile sites were all mechanisms by which legumes incorporated as green manures or harvested as forage (in the case of vetch) would increase labile N and P stocks and improve release of biomass N and P in subsequent cropping.

Conclusions regarding P release from root residues of forage crops might not apply for legumes like lupine harvested as grain, since P from roots might be translocated to shoots during pod fill, altering belowground P stocks. However, Snapp and Lynch (1996) found that P-stressed common bean remobilized P preferentially from leaves and stems to pods rather than from roots, suggesting that reductions in root residue P from pod maturation might be small. Other research confirms a

tendency for a number of legume species to conserve P in root systems under P-limitation (Andrew and Jones, 1978; Kim et al., 2003). Also, higher remobilization to seeds from other plant organs is one strategy of modern breeding for P-efficiency in crops (Horst et al., 1993), which vetch and lupine might not possess since they are crops which have undergone less breeding to maximize bean harvest index. Thus it is plausible that both these species would promote P release from residues to subsequent crops, whether harvested or incorporated in soil.

The fact that simple high/low fertility designations of farmers did not always predict added P impacts argues for knowing specific site factors before promoting RP inputs in heterogeneous soil environments like the Andes. To judge suitability of P addition at a site, soil texture, pH, and plant-available P like Olsen P could be used in place of the more complex dilute HCl-P_i extraction.

However, promoting knowledge of scientific soil predictors for P addition outcomes should not be read as a devaluation of farmer soil designations. Rather, scientific characterization must seek better correspondence with the complexity found within farmer knowledge. For example, low P-fertility, light textured soils where our experiment predicts benefits from P fertilization conform well to farmer typology in our Andean study area. These soils are designated as *thiu jallp'as* (sandy soils) or *challa jallp'as* (shaly soils with abundant rock fragments) in Quechua, and are good soils for legumes by farmers' accounts (Sanchez, 2005). Along with some limited soil testing, these soils form a promising niche for boosting the role of legume BNF as a supporting ecological service using RP or other P sources.

Across the experiment, soluble P fertilizer was the most effective way to improve legume productivity and attributes for nutrient cycling. Nevertheless there is evidence from our experiment and other literature to suggest that the lower impact of RP in the short term might be balanced by other advantages: continued reliance on

AM mycorrhizae, lower cost to farmers, and residual effects of RP placed in the soil. Our experiment did not address residual impacts of RP and TSP or the fates of plant residues. Villaroel (1988) found small residual effects of Capinota RP in high-fertility highland soils, demonstrating that residual effects are plausible in these systems. Measuring longer-term effects of RP, soluble P, and their P-enhanced residues would be promising areas for future research.

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CHAPTER 2

LEGUME FUNCTIONAL ATTRIBUTES AND SOIL FERTILITY CREATE SOIL FERTILITY FEEDBACKS IN AN ANDEAN SMALLHOLDER AGROECOSYSTEM

Abstract

Understanding how plant functional attributes change with gradients in soil type and soil fertility permits better understanding of plants' roles in feedbacks for degradation and restoration of agroecosystems. We used a multi-site experiment to test the importance of species, environmental site factors, and a management-induced phosphorus (P) fertility gradient on legume performance in Andean smallholder farms. Legumes were compared to forage oat, a benchmark grass species for the rotational niche where such legumes might be deployed. Eight of 12 legume cultivars screened had very low biomass and were not adapted in these environments, and adaptation to site factors was important for a given species to have impact via appreciable biomass. However soil P fertility had the strongest impact on legume attributes across species: P uptake, nitrogen (N) fixation, and soil cover were higher at P-fertile sites. Legume biomass N:P ratio was narrower and AM colonization was diminished at P-fertile sites, supporting the trade balance model of legume/AM mutualism. Modulating impacts of soil P on these attributes would likely strengthen feedbacks for degradation or restoration of nutrient cycling. Comparing legumes with oats, N assimilation, P partitioning to roots, AM colonization rates, and residue stoichiometry favorable to N and P release was higher for legumes. Total P uptake and soil cover did not differ between legumes and oats, and oats would make larger contributions to labile soil C pools via C in residues. In low-P fields additional P fertility would be needed to overcome P limitation and make legume attributes favorable for nutrient cycling compared to the oat benchmark species. In comparing two endemic with two

introduced legumes, endemic legumes were more narrowly targeted in their adaptation to site factors, especially elevation, while introduced legumes had good mean performance but high variability caused by poor performance at lower-pH, P-fertile sites. Our results support the concept that a species-specific approach, recognizing soil type and P-fertility differences should be taken in the use of legumes and grasses for soil fertility restoration.

Introduction

In agroecosystems, crop biomass, crop residue quality, and crop-symbiont relationships are important drivers of nutrient cycling. These plant attributes have long been considered important in natural ecosystems. Vitousek (1982) noted that litter biomass:N ratios varied due to nutrient availability across ecosystems, indicating large versus small amounts of nutrients being cycled. Cornwell et al. (2008) concluded that litter quality and biomass are dominant species-specific drivers in decomposition at a global scale. Johnson and others have elucidated the relationship between environmental N:P stoichiometry and plant-mycorrhizal (AM) symbioses (Johnson, 2010; Johnson et al., 2006). Meanwhile, for agricultural ecosystems, Drinkwater and Snapp (2007) reviewed important impacts of plant attributes and diversity on nutrient cycling, and Fiener and Auerswald (2007) demonstrated at a crop rotation scale the impacts of crop attributes and crop sequences on erosion rates.

Considering the range of agroecosystems currently under human management globally, it is likely that crop attributes and their responses to environment and soil fertility are stronger as drivers in low-input smallholder agriculture than in industrial agriculture. Large-scale industrial agriculture is a managed ecosystem where high yields of crops result from strong intervention in nutrient cycles, often with fertilizer inputs, for non-limiting nutrient supplies in soil. In contrast, smallholder farmers in

the developing world often manage crops and soils in marginal economic and geographic settings. They also balance food and forage subsistence and stability with maximum productivity as goals. Nutrient additions in smallholder cropping systems are thus economically limited and usually depend on local primary productivity, and nutrient limitations often constrain crop growth. Under these conditions, plant traits or attributes like residue quality, plant-microbial symbioses, and soil coverage against erosive rainfall are important in sustaining future agricultural production and conservation of ecosystem services (Duran Zuazo and Rodriguez Pleguezuelo, 2008).

Regardless of scale, both industrial and smallholder agriculture usually entail drastic curtailing of plant diversity in agroecosystems. Drinkwater and Snapp (2007) argued that a principal means of improving nutrient cycling is to understand and deploy a greater diversity of crop species and related crop functional attributes to reverse degradation processes and improve nutrient availability and conservation in soils. Low crop species diversity in agroecosystems thus presents an opportunity, allowing for dramatic impacts from addition or rehabilitation of strategically chosen species. These authors focus mostly on nutrient pollution in large-scale agriculture, but potential impacts for greater diversity in soils threatened by degradation in smallholder agriculture are also promising.

One common approach to reversing soil degradation among smallholders that exemplifies this diversity strategy has been the promotion by outside actors of legumes among smallholder farmers. Legumes are promoted because of attributes linked to N fixation, sometimes with insufficient attention to environmental and management effects on these attributes. Legumes contribute to N fertility with N fixation particularly when they are used as green manures. They also enhance P availability to other food and forage crops via transfer of applied and recalcitrant soil P into P-dense residue pools (Kamh et al., 2002). However, in order for farmers to

benefit from legumes in crop rotations, it is essential to understand strengths and limitations of different legume species with regard to soil fertility and environment. One example of good species/agroecosystem fit is alfalfa (*Medicago sativa*), which is grown in temperate agroecosystems worldwide and has large nutrient cycling impacts based on attributes such as wide adaptation to soils and fertility regimes; large biomass potential; substantial, deep and persistent root residues, perennial soil coverage, abundant N fixation, and high shoot forage and residue quality.

To make strategic species choices, research to improve agroecosystem diversity must address three key areas: 1) the adaptation of potential species to a relevant set of farming environments, 2) the response of these species to fertility gradients, and 3) the expected impacts of these species on nutrient cycling.

Expanding on these research goals, species adaptation to environmental conditions is especially important for smallholder farming systems. Soil type and climate heterogeneity is a common feature of smallholder agriculture, especially in mountain regions like those where we focused our research. Because producing appreciable biomass relates to nearly every other plant impact on soils, from soil coverage to relationships with symbionts, species adaptation to environmental variation must be considered alongside the way that management affects traits.

Second, plant attributes will likely respond to soil fertility gradients created in extensive smallholder farming systems. Most smallholder farmers cannot afford purchased fertilizers, and manure produced by livestock is exceeded by the land in annual crops. Scarcity of inputs then leads to fertility gradients based on infield/outfield distinctions (Elias et al., 1998; Konde et al., 2001). Our research tested the response of legume attributes like residue nutrient stocks or AM symbioses to these management-induced fertility gradients. Other work has shown the

importance of analyzing both environmental and management impacts on plant functional traits for restoration (Eviner and Hawkes, 2008; Pakeman et al., 2009).

A third important focus of our research relates to these gradients and legumes' impact on future nutrient cycling. Most interesting in this regard is the way that legumes' response to soil fertility creates feedbacks that degrade or restore future nutrient cycling. For example, adapted legumes with sufficient nutrients (especially P and micronutrients) enhance N availability in agroecosystems over time, increasing productivity of crops across the rotation (Ross et al., 2008). On the other hand, deploying legumes in extremely low fertility environments may in fact hasten degradation if other nutrients are at such low levels that they preclude N fixation and result in low legume productivity. In order to reap the benefits of adding legumes to crop rotations, a minimum threshold of soil fertility is likely required, and our research tested the existence of such thresholds.

In contrast to this N fixation response to general soil fertility (including P fertility), arbuscular-mycorrhizal (AM) symbioses are thought to respond to soil fertility according to the trade balance model (Johnson, 2010). This model predicts mutualism at high levels of soil N:P and parasitism by AM fungi at low soil N:P, which may lead to lower colonization rates as plant allocate resources to other limitations in the presence of abundant P. Legume BNF and AM symbioses are thus likely to behave in opposite ways with respect to soil P fertility gradients: rhizobial symbioses are fostered by low soil N:P, while AM colonization is attenuated.

Responses to gradients in soil fertility by these two symbioses in legumes are important for nutrient cycling because they have consequences for access to N and P and the amounts of these nutrients in residues. The strength of these symbioses also would be expected to carry over into abundance of microbial propagules such as spores and the establishment of these symbioses in future crops. Rhizobial and AM

symbioses could thus help to structure feedback impacts on nutrient cycling via their response to fertility gradients.

Comparing management feedbacks and species attributes

In extensive smallholder agroecosystems, one of our central hypotheses is that feedbacks between management and legume attributes are stronger in their impacts on nutrient cycling than differences in intrinsic legume species attributes and legumes' response to environment. To be sure, species differences in effects on nutrient cycling would be expected between legumes and non N-fixers they replace in crop rotations. However, we expect that among legumes species response to soil fertility will likely play a stronger role than differences among species. The fact that soil fertility creates degrading or regenerating feedbacks and thresholds for legume impact also distinguishes smallholder agriculture from industrial agriculture. In industrial agriculture, direct management of inorganic nutrient pools at non-limiting levels usually bypasses these feedbacks. Understanding the impacts of environment and management on legume attributes is a way of testing this hypothesis, which has particular relevance to smallholder agroecosystems.

Research Questions

Our research seeks to understand the response of legume attributes to farmer nutrient management, soil type, and climate factors in an extensive Andean agroecosystem. As in other smallholder farming systems, Andean crop rotations display standardization because food and forage needs from the rotation are set by nutritional, cultural, and market requirements, with food and forage production assuming primary importance. Subsistence requirements, climate, and agricultural history have led to Andean rotations with a predominance of potato and maize as staple crops that receive manure inputs at the beginning of the rotation. Cereal forages and grains such as forage oats and barley are planted later in the rotation without

manure. Pulses crops such as fava beans and Andean lupine (*Lupinus mutabilis*) are also planted later in the rotation, but not in every rotation cycle. Research questions regarding the intensification of legumes and their attributes in this rotational niche are thus particularly relevant, given the lack of legumes and the predominance of grasses as a benchmark or reference species. Cereal crops and legumes are planted several years after manure addition, when soils are in a depleted phase so that residue quality, N fixation, access to recalcitrant nutrient pools, and microbial symbioses are all important to impacts for future nutrient management.

Within this late-rotation niche, we compared the performance of several legume cultivars (hereafter referred to by species designations) and also measured the impact of management and environmental gradients on these species. To find a relevant set of species, we first screened a larger set of legumes, and then tested hypotheses about the response of better adapted legumes to these gradients in comparison to a benchmark grass species typical of the cropping system. Of special interest in these responses were attributes combining agronomic relevance to farmers and plant species' impacts on biogeochemical cycling of C, N and P.

We characterized variation in response of these four legume species to environmental variables such as soil type and climate. We hypothesized that, as found by Eviner (2004), legumes would differ in adaptation and thus biomass in different environments. We also predicted that attributes related to growth habit or biomass partitioning would differ among species across environmental gradients, since we intentionally chose legumes with a wide variety of origins, crop uses, and life strategies. Furthermore, we predicted that two endemic legume species would differ from recently introduced legumes in having attributes that were more robust to environmental variation across local agricultural settings.

Based on grass/legume contrasts in other ecosystems, we predicted large differences between legumes and a benchmark grass species, in attributes directly related to N fixation such as residue C:N ratio and total N uptake. We also predicted that legumes would take up greater amounts of P at low fertility than the grass, due to legume P uptake strategies geared towards supporting N fixation (Marschner, 1995).

However, despite species-level responses to environment, we hypothesized that a management-induced soil fertility gradient would play a dominant role in modulating legume attributes related to degradation and restoration of nutrient cycling. In other words, legume attributes at low soil fertility, regardless of species, would lead to attributes like soil cover and residue quality and quantity insufficient to prevent further degradation of the system, while high soil fertility would foster legume attributes conducive to restoring ecosystem productivity and enhancing nutrient cycling. This hypothesis was informed by the large range of field productivities and erosion rates we observed directly, and on infield/outfield distinctions expressed by focal groups of farmers (Sanchez, 2005).

Regarding soil N and P fertility effects on symbioses, we expected that an infield/outfield gradient would entail P limitation, and that legume-rhizobial symbioses would respond to higher ambient P fertility across sites with increased proportions and amounts of fixed N. For legume-AM symbioses we hypothesized that for legumes *and* oats, mutualistic interactions at high soil N:P would encourage higher rates of mycorrhizal colonization because of greater resource allocation to the symbiosis. Because fixation of atmospheric N effectively augments the soil N:P ratio for N fixers versus non-fixers, we also hypothesized that mycorrhizal legumes would have higher rates of AM colonization than forage oats.

We expected that soil N:P ratios would relate to the intensity of rhizobial symbioses, though in an opposite way to that predicted for plant-AM symbioses. That

is, high P and low N environments would greatly favor the rhizobial symbiosis of an adapted legume, while making the AM symbiosis less mutualistic. We tested the symmetry of these two symbioses against the soil N:P ratio as a fundamental ecosystem nutrient ratio as suggested by Cleveland and Liptzin (2007).

Materials and Methods

Study area

Hypotheses were tested in experiments over two years (2005-2006 planting seasons) at elevations ranging from 2700 to 4000 meters in the Bolivian department of Potosí (Latitude/ Longitude: 66 º58'W/17 º54'S **to** 66 º15'W/ 18 º15'S). Average rainfall is 650mm, occurring in a rainy season from October to March (FAO, 2010). Mean growing season temperatures ranged from 9.5ºC to 18.0ºC depending on elevation. Soils are dominantly eutric leptosols with some eutric and dystric cambisols in fields with deeper soils (Dijkshoorn et al., 2005).

Screening of legume cultivars

In a first year of research (2005-06), we tested 11 legume cultivars at four sites (three blocks per site) that ranged in elevation from 2650 to 3950 meters above sea level (masl). Cultivars, hereafter referred to by species designations, were Andean lupine (*Lupinus mutabilis* or *tarwi*, locally), lana vetch (*Vicia dasycarpa*), forage pea (*Pisum arvense*), *Medicago polymorpha*, annual white sweetclover (*Melilotus alba*), two varieties of *Lathyrus sativus*, two varieties of *Vicia sativa*, subterranean clover (*Trifolium subterraneum*) and egyptian clover (*Trifolium alexandrinum*). Seed for lupine was acquired locally, while seed of the two *Lathyrus* and *Vicia sativa* cultivars were received from the international nursery trials of the International Center for Agricultural Research in Dry Areas (ICARDA, Aleppo, Syria); all other seeds were purchased from SEFO (Cochabamba, Bolivia). Planting and sampling for these

species on 2-m^2 plots is outlined in Vanek et al. (Chapter 1). For screening purposes in the first year, parameters measured were percent cover, height in cm, shoot biomass and aboveground N uptake (see below). Two legume-grass mixes, oats/vetch and lolium multiflorum/melilotus oficinalis were also included in screening, but results are not presented here.

Legume functional traits related to soil nutrient cycling

Four legume species that covered a range of legume characteristics (weedy/non weedy, native/introduced; mycorrhizal/non mycorrhizal; early/long season) were selected based on the first year of trials. These were seeded in 4-m^2 plots in 2006-07, at 14 different sites (3 blocks per site) bracketing differences in elevation and soil fertility. (details in Vanek, Chapter 1). Lana vetch and forage pea are respectively long and short season, introduced forage legumes, while Andean lupine is an endemic legume that is non-mycorrhizal but has other mechanisms to access unavailable P such as organic acid exudation and proteoid roots (Hocking and Jeffery, 2004; Pearse et al., 2006). Although not tested in the first year, an erect legume weed with local names *arquilla* or *tipa tipa* (*Parocela pacense*) was added in the second year of trials for functional traits because it was observed during a farmer survey to have appreciable biomass. *Arquilla* produces abundant, easily collected seed that is readily scarified with boiling water so that its germination resembles that of a crop plant, making it a potentially viable legume for soil improvement. Forage oats (*Avena sativa*) was included in the second-year trials as a benchmark grass crop and the most common non-legume crop in the late phase of the crop rotation at which trials were conducted.

The four legume species and oats were assessed using four attributes that are important in determining how plant species impact biogeochemical cycling of C, N and P. All of these traits may be impacted by soil fertility gradients and can in turn

affect soil fertility, which makes them relevant for understanding feedbacks in degradation or restoration of agroecosystems:

Shoot and root nutrient stocks (C, N, and P) and residue quality: Shoot and root sampling protocols and nutrient analysis (C and N by combustion, P by HNO_3 digest and Murphy-Riley colorimetric P) are explained in Vanek et al (Vanek, chapter 1). Shoot and root biomass were measured separately to gauge the way that harvest of shoots for forage or grain (in the case of lupine) might affect residue pools for decomposition. C:N, C:P, and N:P ratios were also calculated based on C, N, and P contents for both shoots and roots.

Proportion of biomass N fixed from the atmosphere (%Ndfa) and amount N fixed in roots and shoots (amt Ndfa or Ndfa) was assessed using the ^{15}N natural abundance method outlined by Shearer and Kohl (1986). We used the average ^{15}N signature of non-legume weeds in weedy plots, and chicory (*Cichorium intybus*) as references for soil N uptake (details in Vanek, chapter 1). The difference between total N uptake and amount fixed was assumed to be N taken up from soil (Ndfs).

Percent ground cover by legume species and oats at approximately 10 weeks post-seeding was assessed visually, using cards of different percentage cover of total plot area as a visual guide, and comparison among species within experimental blocks as a check on ratings.

Percent root colonization by AM was assessed on cleared and stained roots with trypan blue according to Koske and Gemma (1989), with details in Vanek et al. (Chapter 1).

Site characterization using soil type, climate, and soil fertility parameters

Sites (agricultural fields) over two years of research were characterized with soil texture parameters (% clay and % sand), soil pH and total C and N content, site temperature (strongly and negatively correlated to site elevation, R=0.98), and the five

most available sequential extraction P pools defined by Tiessen and Moir (Tiessen and Moir, 1993), excluding an initial resin-P extraction. Fields were designated by farmers as either high or low fertility (H or L). In the second year of the experiment, paired H and L sites were chosen in each of seven communities. Site characteristics were analyzed using principal components analysis (PCA) and extraction of rotated factors. Four rotated principle components (PCs) described in detail elsewhere (Vanek, chapter 1) were used to interpret the impact of site variability on legume attributes. In decreasing order of percentage site variation these factors encompassed, these were: PC1, management-induced P fertility differences and soil pH which distinguished high and low fertility fields as stated by farmers (28%); PC2 encompassing soil organic C, N, and organic P pools (27%); PC3 reflecting soil percent clay and sand (18%) and PC4 reflecting a combination of elevation impacts on site temperature, and elevation/management related differences in soil calcium phosphate (Ca-P) pools (14%). Rainfall for sites was used as a covariate in analyses where it was significant, but was not included in the PCA because rainfall varies from year to year while the other site variables are more static. In practice it was useful to disaggregate PC4 into its two component parts, elevation and Ca-P pools, when these were not both significant as main effects, to make results more interpretable.

Statistical analyses

To determine relative ranking of legume biomass for the 11 legume species screened during the first year we used analysis of variance (ANOVA, JMP) at each of four sites. To determine response of four legumes' attributes over two years to site characteristics, we used mixed-model ANOVAs (SAS PROC MIXED, SAS institute, Cary NC) combining 17 experiment sites. Site and block within site were treated as a random effects, and species and site covariates as fixed effects. Real number data was cube-root transformed and percentage data was arcsine-square root transformed where

necessary to satisfy statistical assumptions of equal variance. ANOVA F tests and mean separation via Tukey tests with α=0.05 (SAS PROC Mixed) were used to determine significant species differences. Season rainfall and PC scores were used as covariates to gauge main effects and interaction effects on functional traits between species and factors such as soil P fertility, soil texture, or elevation.

Where interaction effects between species and PC covariates occurred, mean separation was conducted at -1 and +1 standard deviation of the covariate, to assess differential effects of species at a representative range of site characteristics. For rainfall, 600 and 800 mm rainfall were used as covariate levels for mean separation, representing about 75% of the range in rainfall across sites over two years. In the case of soil organic matter (SOM) levels as a covariate (PC2), skewed distribution of site scores for PC2 required mean separation at PC2= +/- 0.5, where most sites clustered, to avoid leverage by two sites on peat-influenced soils with high SOM content.

To analyze stability of performance of functional traits and uncertainty deploying the four legume species, we conducted separate mixed-effect ANOVAs on total N uptake for each species. Among these ANOVAs, we compared the proportion of variance explained with addition of covariate regression terms to a null model with only site and site*block as random effects. Since covariates explained site to site random-effect variation in the model, we concluded that species for which a greater proportion of the site-related variance was absorbed by fixed-effect regression terms were less uncertain in their potential use since their performance was better explained using site characteristics. Conclusions about certainty in use were balanced against overall mean performance across elevation, soil type, and soil fertility niches where a given legume was adapted.

Linear and multiple regression was used to assess the impact of legume biomass, total N and P stocks, and PC covariates on %Ndfa, C:N, and C:P ratios. We

also used regression to test relationships between AM colonization and soil and plant biomass N:P ratios suggested by the trade balance model. Soil N:P in these regressions was denoted by soil $N:P_{inorg}$, or the ratio of total soil N content to the sum of two inorganic P pools (Olsen-P_i and NaOH-P_i) with the strongest correlation with management-induced soil P fertility (PC1).

Results

Legume biomass

In our initial screening, above-ground biomass production of legume cultivars varied by more than 10-fold ranging from 0.1 to 4 $Mg \cdot ha^{-1}$(Table 2.1), and also varied with elevation. Averaged across elevations, lupine, forage pea, and vetch showed the best performance compared to the other legumes in our screening, while *Lathyrus sativus* and *Trifolium alejandrinum* were consistently poor performers. Subterreanean clover, *Medicago polymorpha*, and annual sweetclover grew moderately well at particular sites but were not included in the second year of research. Based on these data, lupine, vetch, and forage pea were used in the second year of experiments. We also added an endemic weedy legume, *arquilla* (*Parocela pacense*) in order to include two legumes that naturally occur in these agroecosystems.

Biomass variation among sites exceeded variation among species, and was driven largely by soil site fertility and elevation. Figure 2.1 shows species biomass means over two years at four elevations, and compares sites classed as low or high fertility by farmers. For biomass averaged across sites for the four species, there was only a 3-fold difference, while site averages for biomass varied by over 14-fold. Elevation and P fertility played a large part in this variation among sites, as seen in the very different elevation trends for the species lupine and arquilla, and the difference between high and low fertility fields between graphs (Fig. 2.1, Table 2.2).

Table 2.1. Mean biomass of legumes at across four sites in an elevation gradient in 2005-2006. Standard error across sites given in parentheses.

Species	Shoot dry biomass ($Mg \cdot ha^{-1}$)
Andean lupine	**4.0** (1.9)
Lana Vetch	**3.5** (1.5)
Forage pea	**2.6** (1.6)
Subterranean clover	**1.6** (0.5)
Medicago polymorpha	**1.1** (0.4)
Vicia sativa ICARDA-2	**0.7** (0.2)
Vicia sativa ICARDA-1	**0.7** (0.1)
White sweetclover	**0.5** (0.3)
Egyptian clover	**0.3** (0.1)
Lathyrus sativus ICARDA-1	**0.1** (0.0)
Lathyrus sativus ICARDA-2	**0.1** (0.1)

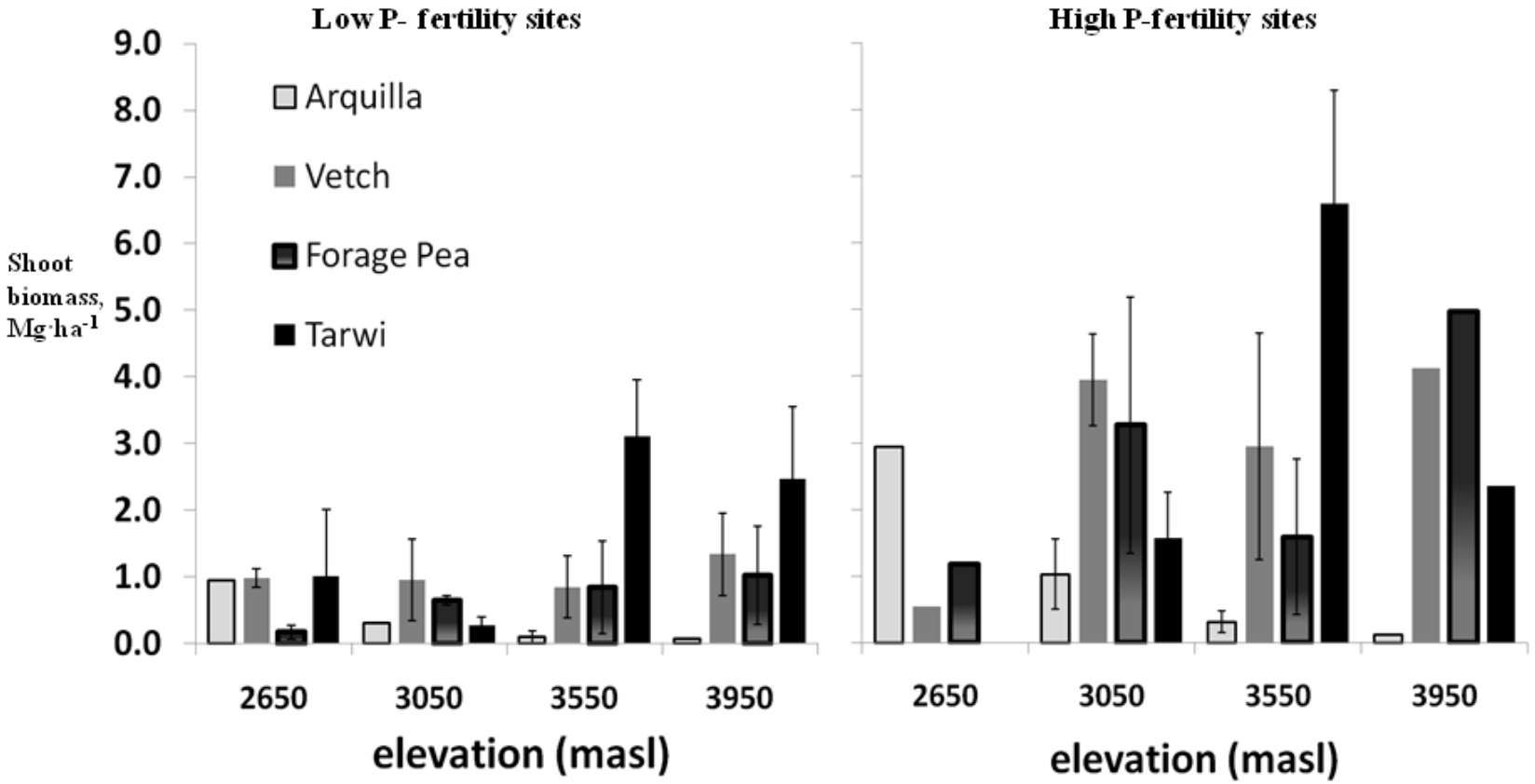


Figure 2.1. Shoot biomass of four legume species at low soil fertility (left) and high soil fertility (right) sites. Each data point is the mean biomass from between 1 and 3 sites (3 replications per site) over two years at the elevation indicated. For *arquilla* only one year of data was obtained.

Table 2.2. ANOVA results for biomass, total root+shoot N, and Ndfa of forage oats and four legumes. PC 1 through 3 are rotated factors derived from a principal components analysis (PCA) of site characteristics. For clarity in the interpretation of elevation and calcium-P pools, PC4 from the PCA was separated into site temperature (elevation) and ln(DHCl-P_i), the calcium phosphate pool from soil analysis, when one or the other was shown to be non significant in the model.

Species parameter:	Shoot Biomass		Total N uptake (roots + shoots)		N fixed (Ndfa)	
Model term	**F**	**p-value**	**F**	**p-value**	**F**	**p-value**
Species (spp.)	**64.6**	**<.0001**	36.7	**<.0001**	35.9	<.0001
Main covariate effects **(sign of parameter given for significant main effects)**						
Elevation (site temperature)	0.1	ns	0.0	ns	0.6	ns
Site Rainfall	ns	ns	ns	ns	0.1	ns
PC1: Soil P fertility	6.6	**0.01 (+)**	4.9	**0.03 (+)**	6.4	**0.02 (+)**
PC2: Soil organic C, N	ns	ns	ns	ns	ns	ns
PC3: Soil texture	0.1	ns	0.4	ns	0.0	ns
Soil dilute HCl-P (Ca-P pool)	ns	ns	ns	ns	ns	ns
Spp. x covariate interaction effects						
Elevation x spp.	20.9	**<.0001**	15.9	**<.001**	13.4	**<.001**
Rainfall x spp.	ns	ns	ns	ns	ns	ns
PC1, P fertility x spp.	2.0	0.10	3.1	**0.02**	5.9	**0.001**
PC2, Soil organic C,.N x spp.	ns	ns	ns	ns	ns	ns
PC3, soil texture x spp.	6.5	**<.0001**	6.2	**<.001**	3.8	**0.01**
Soil dilute HCl-P x spp.	ns	ns	ns	ns	ns	ns

The farmer distinction of high and low fertility was best correlated to variation in soil P fertility and soil pH (PC1) from the PCA (Vanek, chapter 1). Soil P fertility (PC1) also had significant main and interaction effects with species on crop biomass in an ANOVA on biomass data (Table 2.2), and elevation and soil texture (PC3) had significant interaction effects, indicating direct and differential impacts of these gradients on crop productivity. Fertility, elevation, and soil type effects on biomass are important because biomass either directly impacts or must be considered alongside other plant attributes that generate feedbacks in degradation or restoration of agricultural systems (Fig. 2.1).

Percent ground cover by crops

Results for soil coverage at mid-season paralleled trends in productivity and other plant attributes that affect nutrient cycling, and higher soil fertility fostered higher soil cover. Since biomass and plant growth habit determine a species' soil coverage, the correspondence between percent cover and final biomass at harvest is not surprising. Just as for biomass there was a positive main effect of soil P fertility on percent ground cover (+7% cover increase per unit increase in the normalized PC1 covariate), which translates to an increase of 20% in percent cover over the range of P fertility encompassed by experiment fields (+/- 1.5 std deviation in PC1) (Table 2.3). Higher soil cover in P-fertile soils was superimposed on differences among species: vetch, forage pea, and oats had higher percent cover than lupine or arquilla.

Soil organic matter content (SOM) and soil texture had differential impacts on species' midseason ground cover. Higher SOM was associated with increased cover for vetch, forage pea, and oats, while lupine and arquilla did not increase (interaction PC2 x species, $p=0.02$). Heavy soil texture favored soil cover of forage pea and vetch over lupine, arquilla, and the forage oat control, while oats had highest soil cover in light-textured soils (interaction PC3 x species, $p=0.002$).

Table 2.3. Direct and differential impact of soil P fertility (PC1) on species' percent ground cover, total P uptake, shoot and root biomass C and residue quality.

	Percent ground cover	total biomass P ($kg \cdot ha^{-1}$)	Whole biomass N:P	Shoot: root biomass C (ratio)	Shoot C:P	Root C:P	Shoot C:N	Root C:N
Significance p for main effect, PC1	**0.03***	**0.05***	**<0.01****	**0.21^{ns}**	**0.02***	**<0.001****	**0.03***	**ns**
Significance p for interaction, PC1 x Species	**ns**	**0.002****	**ns**	**ns**	**<0.001*****	**ns**	**ns**	**ns**
	Low P fertility (PC1 = -1)							
Tarwi	16 b	3.4 b	16.6 a	1.3 c	352 ab	394 b	14.2 c	24.2 c
Arquilla	9 b	1.4 c	8.1 c	0.8 d	167 d	253 c	17.1 b	33.3 b
Forage Pea	33 b	3.4 b	17.2 a	1.8 b	300 bc	253 c	17.4 b	17.1 d
Vicia	38 a	5.8 a	14.1 b	1.4 c	243 c	290 c	14.8 c	19.9 d
Forage Oat	*38 a*	*6.6 a*	6.0 c	2.3 *a*	*410 a*	*559 a*	62.6 *a*	72.6 *a*
	High P fertility (PC1= +1)							
Tarwi	28 b	9.9 a	12.1 a	1.8 c	175 b	264 b	16.6 c	22.2 c
Arquilla	18 c	3.4 c	5.4 c	1.1 d	105 c	158 c	19.9 b	31.8 b
Forage Pea	47 a	4.7 b	12.5 a	2.4 b	378 a	158 c	20.3 b	18.5 d
Vicia	52 a	6.5 b	10.0 b	1.8 c	183 b	185 c	17.3 c	17.0 d
Forage Oat	*52 a*	*11.3 a*	3.8 c	3.0 *a*	*224 b*	*392 a*	70.4 *a*	61.7 *a*

Percent cover levels in Table 2.4, and effects of soil fertility and texture, are relevant to feedback impacts on soil degradation because they straddle 30%, a threshold where soil cover begins to reduce soil erosion(Duran and Rodriguez, 2008).

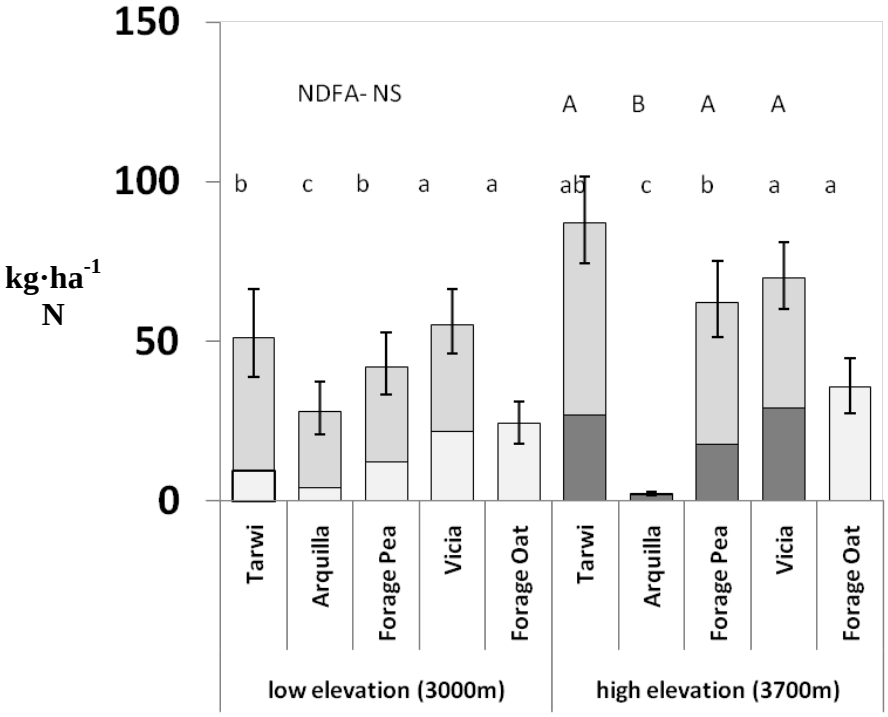
N stocks and N fixation

Total N stocks and amount N fixed (Ndfa) were highly correlated to biomass, with R=0.92 for both total N and Ndfa regressed on biomass ($p<0.0001$). Elevation and soil texture effects differed among species for total N and Ndfa (Table 2.2, interaction $p<0.0001$ for both). Arquilla grew poorly at high elevations, while other legumes increased in both Ndfa and Ndfs from low to high elevation (Fig. 2.2a). Texture also had differential impacts: in light textured soils lupine had higher Ndfa than vetch, while in heavy textured soils vetch had higher Ndfa and Ndfs than either lupine or forage pea (Fig. 2.2c; soil percent clay range: 18 to 47%).

Consistent with our hypotheses, total Ndfa and biomass N were greater at higher soil P fertility sites (high PC1, Table 2.2). Interestingly, no such association was seen between higher soil P fertility and soil-derived N across species, but only a weakly significant main effect ($p=0.09$) of PC2 representing soil organic C and N pools (ANOVA not shown), consistent with SOM's (PC2) significant correlation to N uptake by the forage oat reference crop ($R=0.40$, $p=0.02$).

P fertility also had strong species-specific impacts on N fixed by legumes and total N uptake (Table 2.2). Increases in Ndfa with higher P fertility were greater for lupine than for vetch or forage pea, suggesting strong P limitation of N fixation in lupine as well as the ability to respond in fields with higher P fertility because the endemic lupine was not constrained by other environmental factors (Fig. 2.2b). Furthermore, for lupine the increase in Ndfa was due both to increases in total N and in the proportion of this total that was fixed (Fig. 2.3). Figure 2.3 also shows that

2a.



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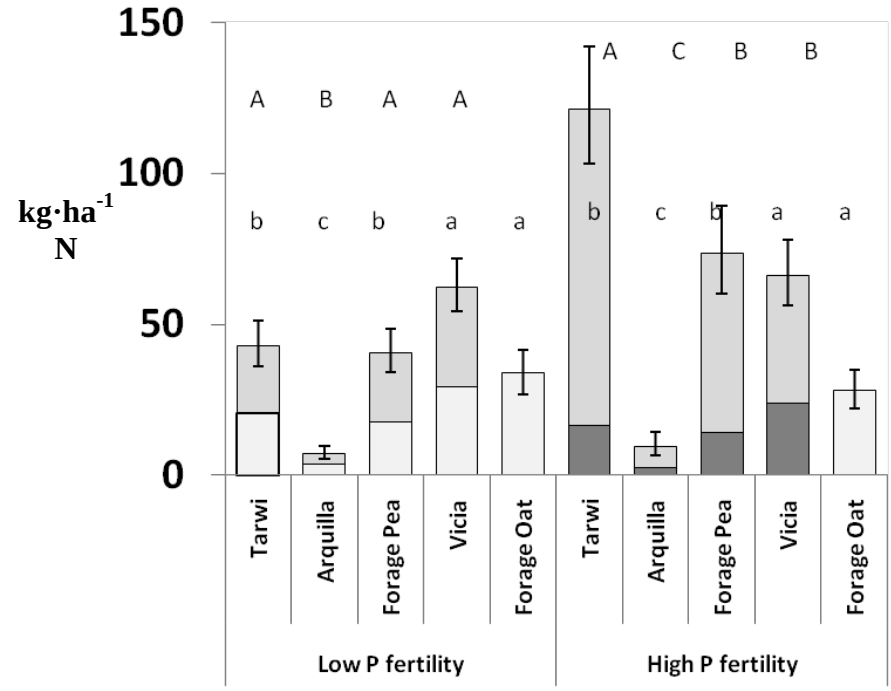
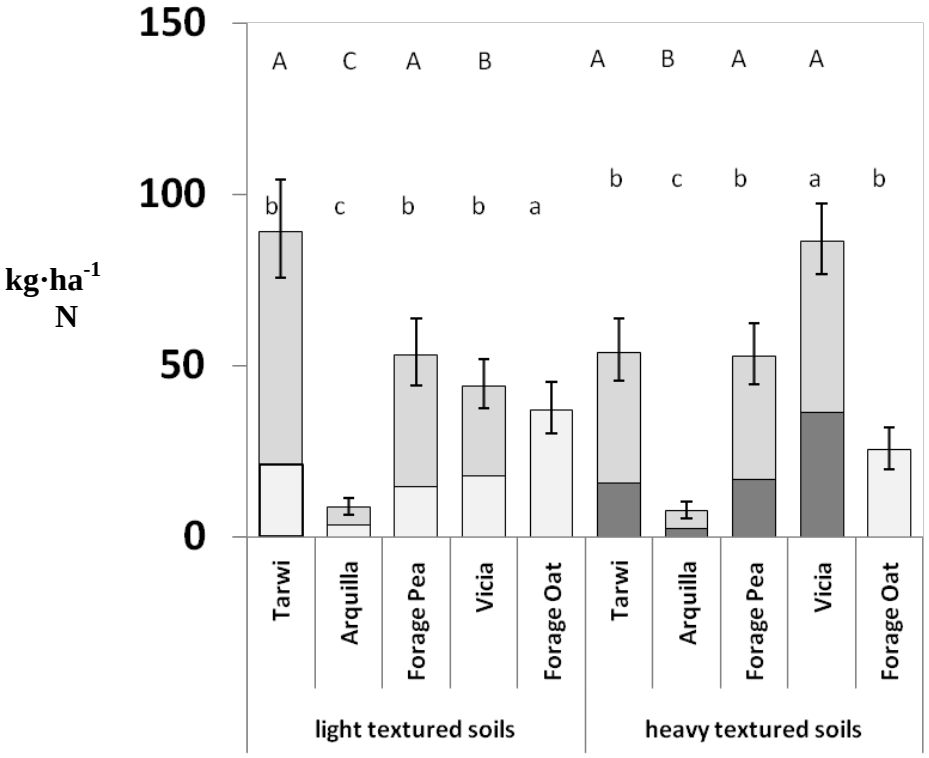


Figure 2.**2a-c. Ndfa and Ndfs for four species and oats with respect to differences in sites in a. elevation (site temperature); b. Soil P fertility and c. soil texture.** Top darker section of bars is fixed N (Ndfa); bottom light section is N from soil (Ndfs). Capital letters denote statistically equal means for Ndfa; lower case letters denote statistically similar means for Ndfs. NS= no significant differences

Figure 2 (continued).

2c.



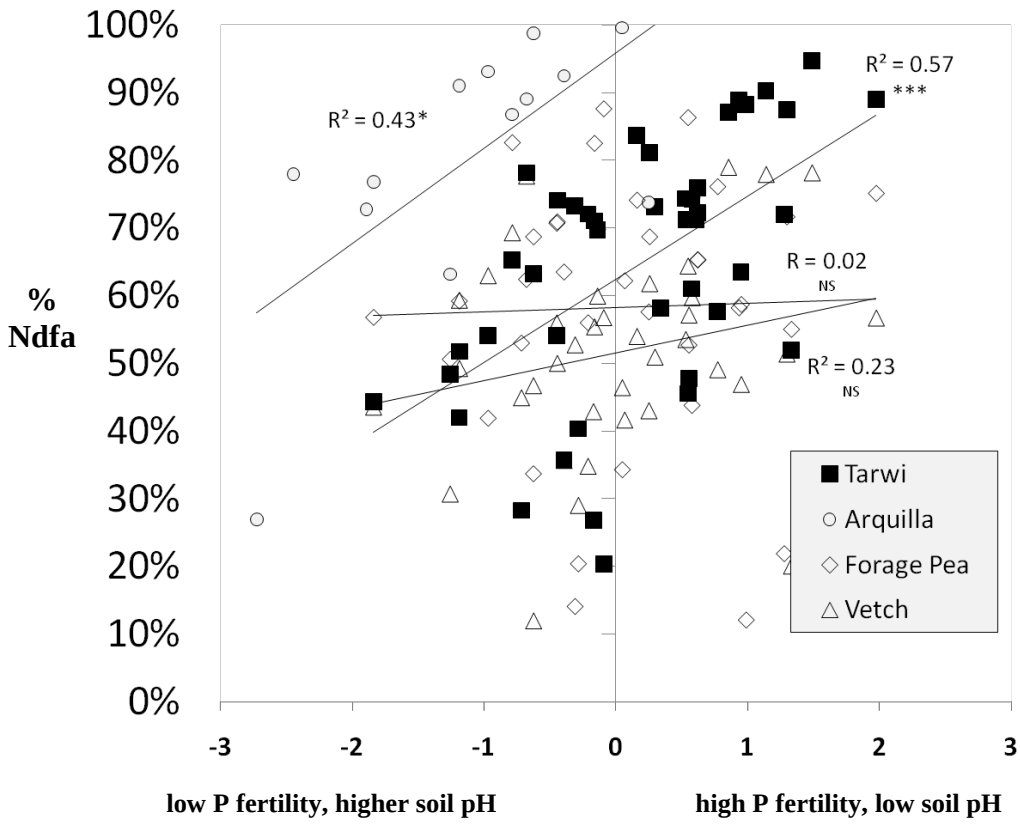


Figure 2.3. Relationship between soil P fertility (values for PC1 from PCA), and %Ndfa for 4 legume species. For arquilla, only warm temperature sites below 3100 m elevation were plotted, since biomass was minimal above this elevation. Correlations are significant for tarwi and arquilla, non-significant for forage pea and vetch.

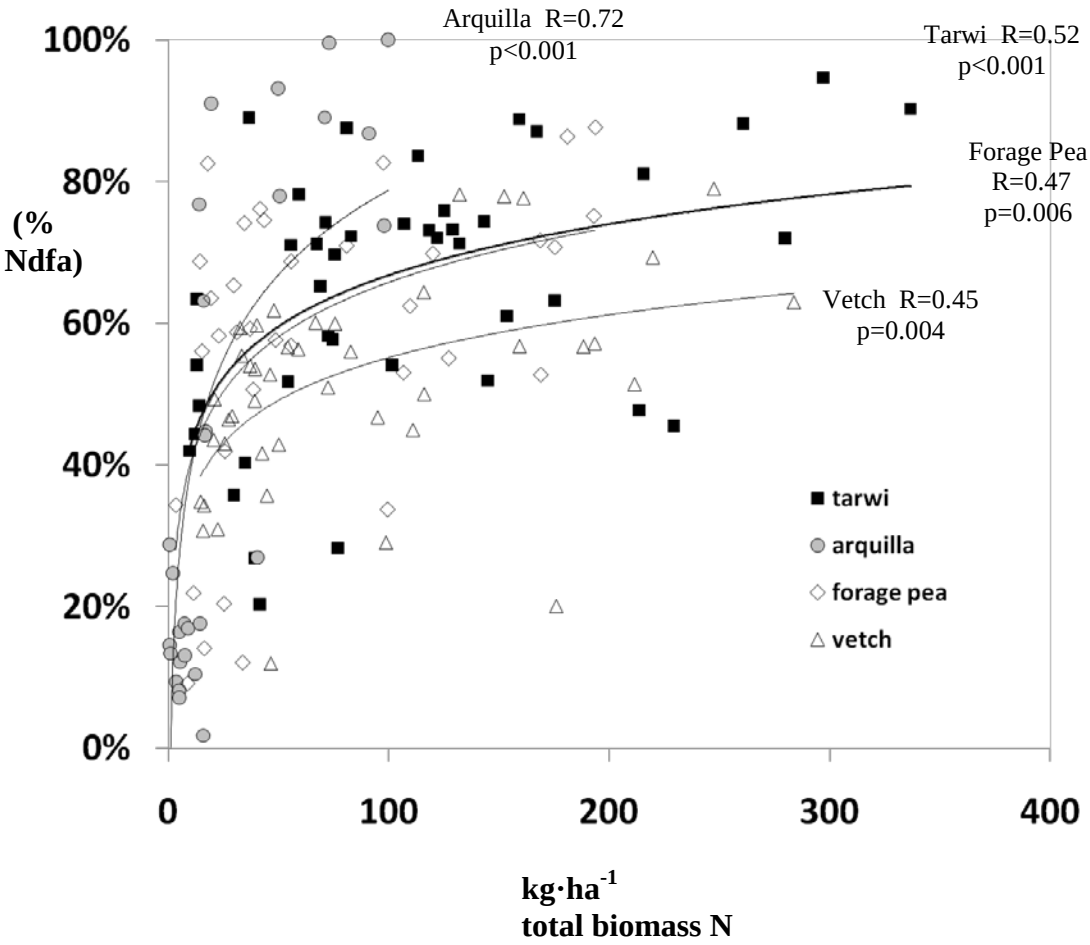


Figure 2.4. **Relationship between percent N fixed (%Ndfa) and total N uptake of legumes.** Fit R and p-values were calculated using log-transformed total N and transformed percentage data in JMP.

%Ndfa for arquilla increased at higher P fertility in sites below 3100 m where it grew well. Vetch and forage pea showed small increases in amount Ndfa with P fertility but not the proportion fixed.

The relationship of biomass to proportion N fixed:

In our experiment, biomass and total N assimilated (soil+BNF) were linearly correlated, while proportion N fixed showed a more complex relationship to biomass. Figure 2.4 shows the relation between proportion N fixed (%Ndfa) and N assimilated as a biomass proxy, indicating limitations on N fixation at low levels of biomass. N uptake was a useful proxy for biomass in this graph because it is relevant to possible BNF depression at high soil N levels. At low levels of N uptake, %Ndfa is variable and lower, while at higher values of N uptake, %Ndfa saturates at values $\geq$ 60% depending on species. An overall logarithmic fit to %Ndfa was significant, as were the different species trends seen in Fig. 2.4 (overall fit $F=35$, $p<0.0001$).

Thus at values of total N uptake above about 70 $kg \cdot ha^{-1}$, the proportion of N fixed remained relatively constant while both N uptake from soil and N fixed increased along with total biomass. There is thus no evidence that high levels of soil N depressed N fixation. Meanwhile when N uptake was less than 70 $kg \cdot ha^{-1}$, the proportion of N fixed declined, suggesting impairment of N fixation, perhaps by P limitation, drought, inappropriate species/temperature match, or disease. Regardless of cause, stunted legumes were not efficient N-fixers. Third, there was overall higher %Ndfa (but lower overall N stock) for the endemic legume weed, and lower %Ndfa (thus higher %Ndfs) by vetch. A mixed model ANOVA confirmed species groupings depicted by Fig. 2.4: [arquilla > (lupine, forage pea) > (forage pea, vetch), Tukey test, $p<0.05$], when cool high elevation sites at lower left of Fig. 2.4 are ignored for arquilla (ANOVA table not shown).

Comparisons between endemic and introduced species

We hypothesized that two endemic legumes, lupine and arquilla, would have more robust or stable functional traits for nutrient cycling compared to two recent introductions. The overall performance of endemic legumes in was in fact more variable across environmental and management gradients, but also more predictable when related to particular niches, rather than being stable over the entire set of environments. Above we showed that two endemic cultivars generally fixed a greater proportion of N with the exception of the very low-biomass sites for the weedy species, and %Ndfa was more responsive to P fertility levels. However, adaptation to particular environments (lupine at high P fertility and high elevations, arquilla at low elevation), rather than broad adaptation, was important to the performance of the two endemic species. This is shown in Table 2.4 comparing variance components in a null model without site covariates to a model that includes significant fixed effects like soil fertility or elevation. We used total N uptake as a proxy variable for legume performance in these models. Because principal components based on site parameters encompassed almost all environmental variability among sites (87% variance in PCs 1-4, see Vanek, Chapter 1), we interpreted the explanatory power of these models as predictability of performance for these legumes. For vetch and forage pea, two recently-introduced species, site predictors absorbed less than 20% of residual site-level variation. For the two endemic legumes, site-level variance could be halved with explanations such as elevation and P fertility. This suggests that endemic species, through a number of mechanisms not investigated here (relationships to rhizobial flora, pest resistance, drought and heat adaptation, etc.) responded in a more predictable way to environmental gradients in expressing functional attributes.

In contrast, at a number of high-fertility, mid-elevation sites, vetch and forage pea were surprisingly unproductive in the second year of trials, muting the increases

Table 2.4. Percent of site- and block-level variance in total N assimilated (fixed + soil N) explainable with site covariates, indicating stability of performance of different legume species at sites like those in the experiment. Species with higher percent reduction in the random effect variance had performance that was better explained by site predictors and thus more reliable.

Species	Null model random effect variance component	Variance component with significant fixed effect terms	Significant fixed effects	Percent reduction in random effect variance
Tarwi	1.57	0.80	**Elevation, P fertility (PC1)**	49%
Arquilla	1.24	0.45	**Elevation, Soil organic C and N (PC2)**	64%
Vetch	1.38	1.13	**Soil texture (PC3)**	18%
Forage pea	1.36	1.19	**Elevation, P fertility (PC1)**	13%

we expected from higher soil P fertility for these legumes. This might have been due to undiagnosed herbivory from nematodes, possibly linked to higher cropping intensity at fertile sites. Poor performance of these crops under both low and high P-fertility could have also resulted from dry periods during the relatively dry second year that were described by collaborating farmers at high fertility sites. It is also possible that forage peas and vetch encountered pH limitations at high fertility sites, since P-fertile sites at middle to high elevations also had low pH (pH and soil P were negatively correlated in the PCA). Neither amount nor proportion of N fixed was higher for vetch and pea at low pH, high-P sites (Figs. 2.2 and 2.3). Meanwhile lupine fixed substantial N even at a soil pH of 4.7. We cannot precisely explain reasons for poor performance of these recently introduced legumes, but disease, drought or pH vulnerability could add risk to the deployment of pea and vetch and limit their contributions to nutrient cycling. However, in spite of greater unexplained variability, mean values for pea and vetch N or P assimilation did not differ from lupine, an endemic crop legume, and exceeded that of the weedy legume, *arquilla* (Fig. 2.2).

Legume comparisons to forage oats: N and P stocks and partitioning

We hypothesized that legumes would aid in improving nutrient status of low-fertility soils by accumulating larger amounts of N and P in residue pools than the forage oat benchmark crop. N assimilation by legumes was indeed higher than oats, but P uptake was lower or equal than for oats. P fertility was once again a significant driver of these attributes. Ignoring interaction effects, mean total N of the three crop legumes was higher than that of forage oats (contrast of (lupine, forage pea, vetch) vs. forage oats, $p<0.0001$), while oat N uptake generally exceeded total N uptake by the weedy legume *arquilla* across the experiment. Interaction effects between species and site covariates (Fig. 2.2) show that vetch had higher total N uptake than oats in low P fertility soils, and lupine exceeded oat N uptake in light texture soils (about 20% clay,

in our set of fields). In heavy textured and high-P soils, crop legume exceeded oat N uptake.

Our experiment meanwhile does not support the hypothesis that these legumes assimilate more P under low soil fertility than non-legumes and thus transfer recalcitrant soil P to labile organic matter pools. Considering the low to high fertility gradient in Figure 2.5, forage oats shared first rank in P uptake with vetch at low fertility and lupine at high fertility. This pattern was repeated in the case of the other environmental gradients considered in our experiment: for example, lupine and oats shared first rank in light textured soils, while vetch and oats shared first rank in P uptake in heavy textured soils (data not shown).

The fact that oat total P uptake equaled or exceeded that of legumes could be due to the fact that forage oats have been bred to produce biomass for livestock, and thus substantial uptake of any given nutrient. Other non-N fixers like wheat, barley, or weeds that tend to occupy late rotational niches in this system might produce less biomass and thus have lower P uptake. Our results show mixed support for this conclusion. Wheat was included as a benchmark grass species in the initial screening experiment, and at two high fertility sites P uptake by both vetch and lupine exceeded that of wheat, supporting the idea that cereal grain crops might differ in comparison to legumes. However in the second year, weeds in reference plots for the ^{15}N method took up similar amounts of P to the three crop legumes as a group, and less P than oats or the best-performing legumes (Fig. 2.5).

Although total P assimilation did not differ between oats and legumes as a group, legumes partitioned a greater proportion of P to belowground structures (taproots, fine roots, and nodules) than did oats (shoot:root P ratio, contrast of oats vs. legumes, $F=43.5$, $p<0.0001$). Additionally, absolute belowground P for vetch

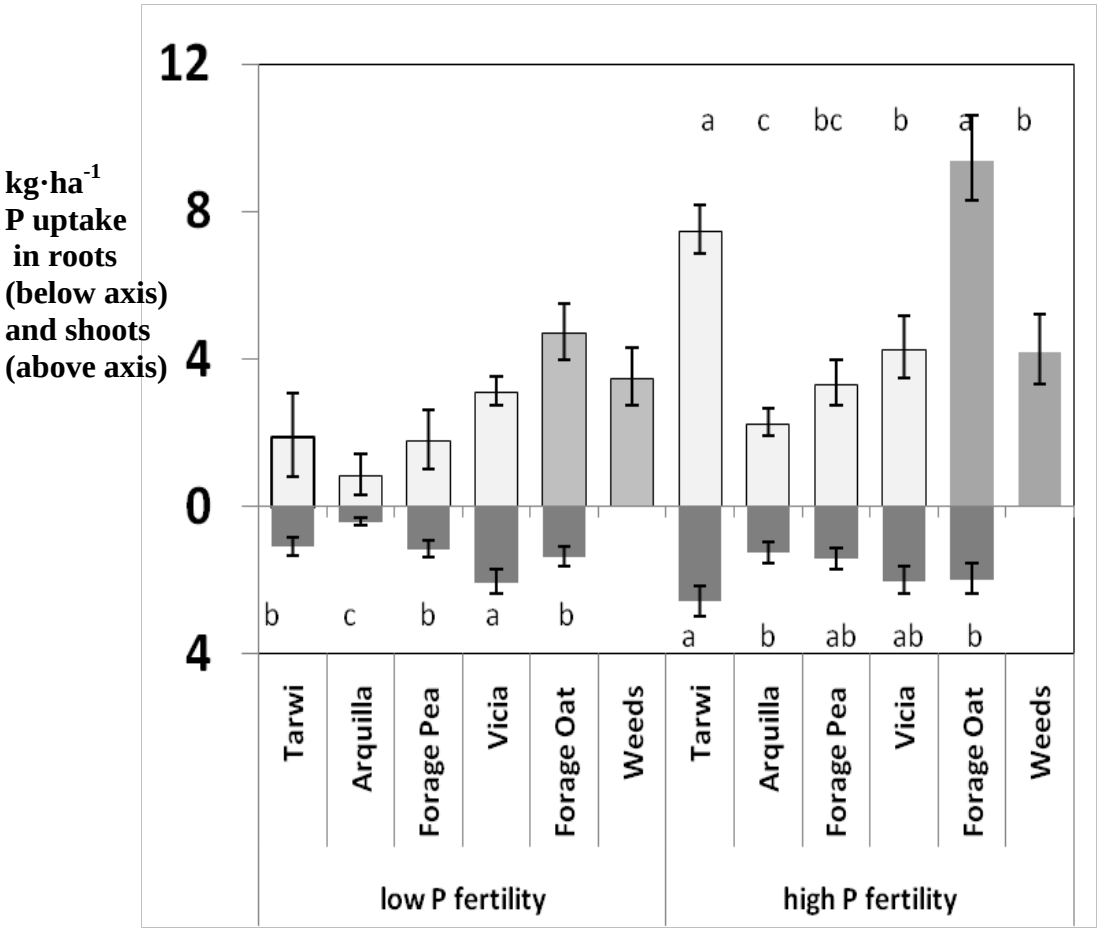


Figure 2.5. Aboveground (above axis) and belowground (below axis) stocks of P for legumes, oats, and weeds. No belowground biomass was measured for weeds. Means with different letters are significantly different at the 5% level using a Tukey HSD test.

exceeded that of oats at low soil P (PC1) and was greater for lupine than oats at high soil P fertility (Fig. 2.5). These differences reflect allocation of P to nodules by legumes and higher P contents of fine roots (Table 2.3, root C:P ratios) If shoot biomass is harvested for grain and forage, as occurs for crop legumes in smallholder agriculture, small additional belowground P stocks paired with robust differences in P partitioning between oats and legumes would likely increase levels of labile P in residue pools.

Residue quality: C:P, P use efficiency, and C:N of biomass fractions

Shoot and root residue stoichiometry (C:N and C:P) revealed three general trends. First, as expected crop biomass had wider C:P ratios at lower soil P fertility in almost all cases. Second, C:P and C:N ratios for oat shoots and roots were both wider than for legumes. Last, residue C:N quality showed little response to soil P fertility (PC1) and SOM (PC2), and the small differences in C:N were thus unlikely to drive differences in nutrient cycling when compared to the large differences in C and N stocks in residues with soil P fertility.

Residue quality of biomass fractions should be considered in light of the fact that higher biomass associated with increased soil P fertility was evenly partitioned to belowground structures and shoots, rather than redistributing from roots to shoots in accordance with plant functional equilibrium[1]. The ratio of biomass C in shoots to belowground structures (taproots, fine roots, nodules) showed only a non-significant increase with either P fertility (PC1) or SOM (PC2, Table 2.3). Considered across soil fertility gradients, increasing C stocks in root residues would thus likely have similar but smaller impacts as would whole-plant biomass.

[1] However, dividing belowground biomass between taproots and fine roots, the C ratio (shoot + taproots: fine roots) increased with P fertility, as expected from plant functional equilibrium theory.

Higher soil P fertility was associated with generally narrower C:P ratios in shoots, with some variability around this trend indicated by significant main and interaction effects of PC1 (Table 2.4). Narrowing of C:P ratios was especially strong for lupine and forage oats, which narrowed by half their shoot C:P going from infertile to P-fertile sites. Forage pea was the only exception to this trend, *widening* its shoot C:P at higher soil P fertility (PC1), perhaps related to the fact that it is as an early-season forage crop with high P use efficiency in producing biomass.

In contrast to results for shoot C:P, the narrowing trend at higher P fertility for root C:P was consistent for all five species (Table 2.3). Averaged across species, root C:P was about 30% lower at high than low soil P fertility (PC1 = +1 vs. -1). This main effect was superposed on species differences: forage oat had consistently wider root C:P ratios than legumes, and lupine exceeded other legumes in root C:P. Species differences in root C:P, and the large main effects of P fertility on this attribute, exemplified the way that soil fertility had a modulating effect on legume attributes.

Results for whole-plant C:P ratio, also designated as a plant's P use efficiency, reinforced that P fertility was a dominant, modulating gradient for plant attributes, and also that oats and legumes had contrasting functional roles in the rotation. This can be seen in Figure 2.6 showing the relationship between whole-biomass C:P and total P assimilation for all plots in the experiment. Phosphorus uptake on the x- axis is a measure of plant-available P and related to soil analyses of P fertility from the PCA (P uptake to PC1 correlation: $R=0.42$, $p<0.0001$). Wider biomass C:P occurred at low levels of P uptake, suggesting that smaller P stocks were associated with less P-rich residues across species in response to lower P fertility. Additionally, forage oats would likely outperform legumes in adding carbon to residue pools due to higher P use efficiency, in the same way that it produced greater biomass of forage (albeit of lower quality) than legumes. For example, oats' fine root biomass C was also 60%

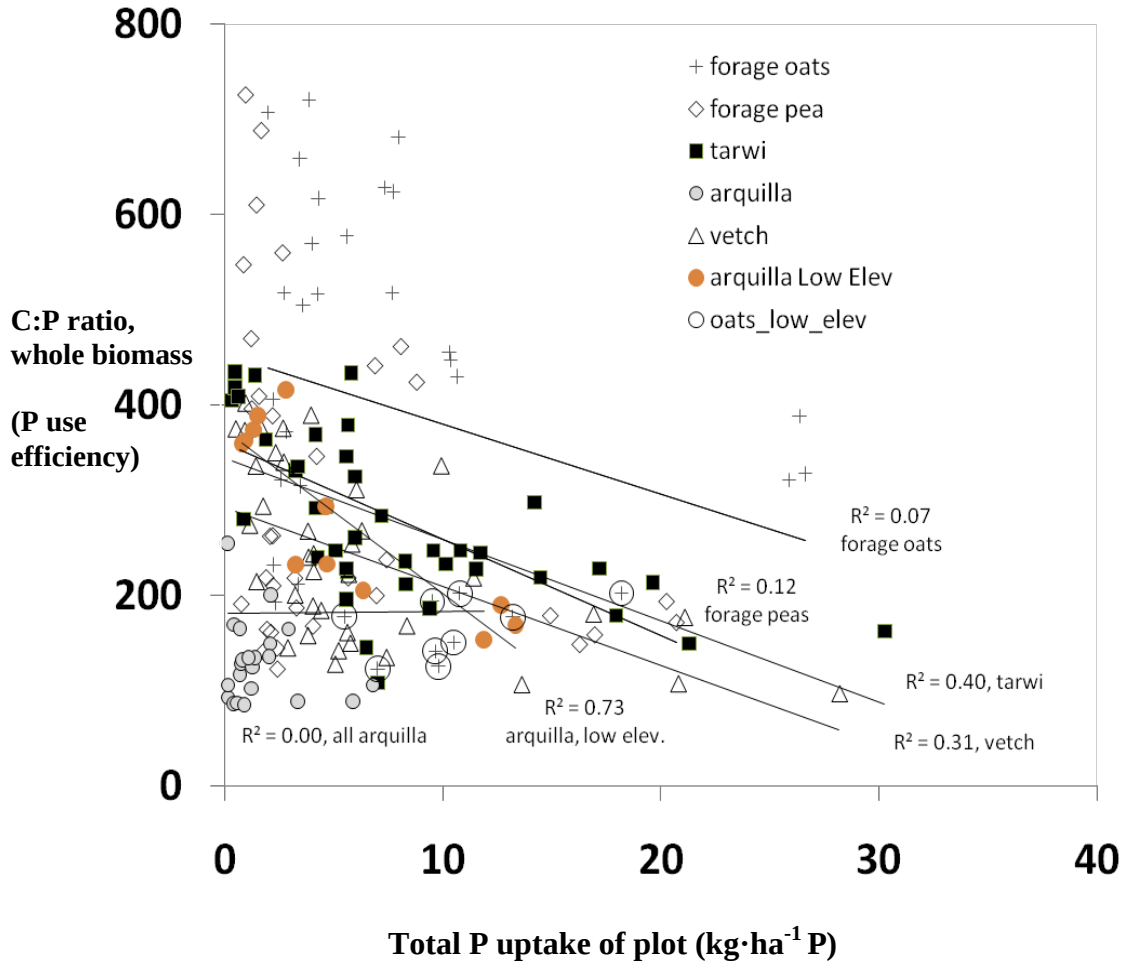


Figure 2.6. Graph illustrating increased P use efficiency and reduced residue quality at lower levels of total P uptake. Legumes are also generally lower than oats in P use efficiency. Narrow values of C:P for arquilla at lower left, and circled symbols for forage oat in the middle range of P uptake likely demonstrate temperature limitation of arquilla at high elevation and drought limitation of forage oats.

greater than that of lupine at high P fertility (PC1), and 75% greater than that of legumes in the grand mean over all covariates (data not shown).

Differences in C:N residue quality of biomass fractions were small. At high soil P fertility levels (PC1) root C:N was slightly narrower, and shoot C:N slightly *wider*. Less N-dense shoots were surprising given increases in Ndfa at higher P fertility shown above, but may be understood as an equal effect of P fertility on overall biomass as on BNF, and thus dilution of fixed and soil N in biomass. Meanwhile, shoot and root C:N ratios decreased by less than 10% in fields with higher organic C and N (PC2). For example shoot C:N averaged over legume species decreased from 17.6 to 16.3 for a range in SOM that captured most sites (PC2= -0.5 to +0.5). In contrast to the large differences in C:P ratio, C:N ratio differences with soil P fertility and SOM content were minor, similar to differences in C:N among legume species (Table 2.3), and smaller than the differences in C:N between legumes and forage oats.

N:P stoichiometry, plant-AM and plant-rhizobial symbioses.

We hypothesized that AM colonization of legumes would exceed that of forage oats, due to higher biomass N:P ratios in legumes linked to BNF. Our results supported this hypothesis, with AM colonization rates ranked as forage pea > (arquilla, vetch) > forage oat. Ranking of legume species varied slightly with soil P fertility (interaction of species x PC1, p=0.005), but forage oat always remained below legumes in these rankings. Most colonization rates were above 50% (Fig. 2.7), suggesting that AM fungi play an important role in these agroecosystems. We confirmed that Andean lupine is non-mycorrhizal, with rates of colonization zero at most sites and less than 10% in a few plots.

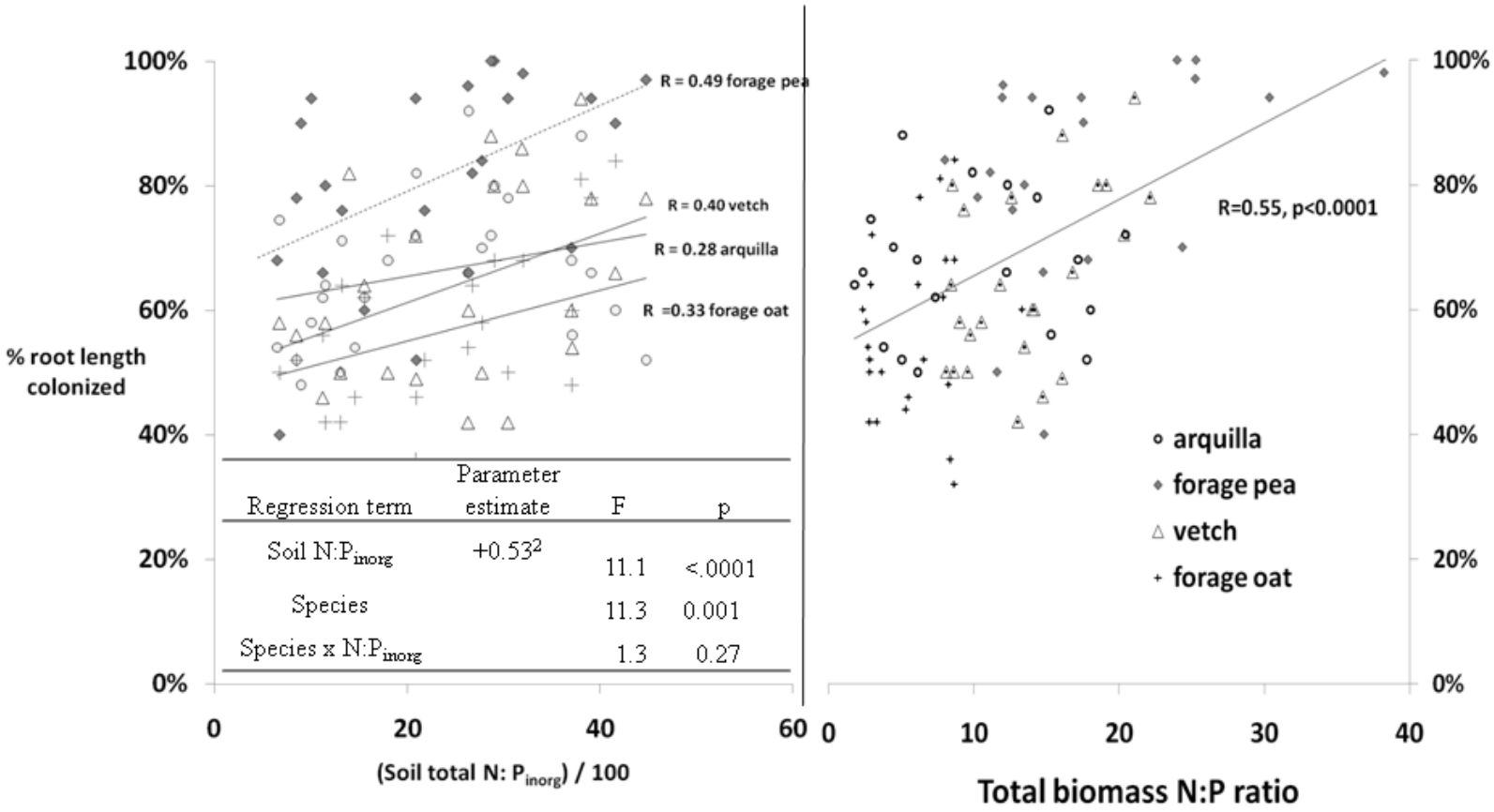
As we had predicted based on the trade balance model, there was a significant and positive relationship between AM colonization of mycorrhizal species and soil $N:P_{inorg}$ (Fig. 2.7a). The species ranking of AM colonization is also apparent in this

graph, with oat colonization lagging that of the three legumes. Soil $N:P_{inorg}$ was also predictive of total biomass N:P of the four mycorrhizal crops across species [R=0.67, p<0.0001]. Total biomass N:P differed among species in roughly the same order as AM colonization (Table 2.3, right), and was predictive of AM colonization across species, which suggested to us a significant single linear relation between whole biomass N:P of the four mycorrhizal species and AM colonization (Fig. 2.7b). This linkage between soil nutrient stoichiometry, plant N:P ratios related to P limitation, and mycorrhizal symbiosis supports the trade balance model and suggests that it is applicable to agroecosystems.

Compared with the robust relationship between soil N:P and AM symbioses across species in these field sites, soil N:P was a weak predictor of the intensity of legume-rhizobial symbioses. We found the expected decrease in %Ndfa with increasing soil N:P for lupine (R=-0.42, p=0.004) and for arquilla at warm sites where this weed was adapted (R= -0.70, p= 0.002). However this relationship was absent for forage pea and vetch, probably because of the soil pH or other limits to these species already described. These results show the same species pattern as the response of %Ndfa to soil P fertility (PC1; Fig. 2.3), suggesting that P_{inorg} was likely the driving factor within the $N:P_{inorg}$ ratio for %Ndfa. This conclusion is also supported by the constantly increasing trend of %Ndfa with N uptake in Fig. 2.4, indicating that high soil N fertility did not depress amount N fixed or %Ndfa.

Discussion

Our research sought to measure response of legume attributes related to biogeochemical cycling to soil and climate characteristics, as well as management-induced gradients of soil fertility. Mapping these responses can help to plan strategic species and management choices that maximize positive impacts of increasing crop



Regression term	Parameter estimate	F	p
Soil N:P_{inorg}	+0.53[2]	11.1	<.0001
Species		11.3	0.001
Species x N:P_{inorg}		1.3	0.27

7a. % AM colonization vs. Soil N:P_{inorg}

7b. Percent AM colonization vs. total biomass N:P

Figure 2.7 a,b. AM colonization plotted as a function of soil N:P_{inorg} ratio(left) and total biomass N:P (right) for three legume species and forage oats. Scale for N:P_{inorg} of soil has been divided by 100. Regression table for AM colonization given at left.

[2] Equivalent to a 0.53% increase in root colonization per unit increase in the abscissa (soil N:P ratio/100).

diversity in smallholder cropping systems. Our conclusions focus on four areas: the importance of species adaptation and species specificity to environment; the way that soil fertility modulates attributes to create restoring or degrading feedbacks in agroecosystems; consequences of soil fertility for two major legume symbioses; and crop choice and management implications of these findings. In these conclusions we distinguish proximal impacts caused by response to the environment (biomass, nutrient stocks, percent cover, etc.) from longer term impacts and feedbacks (from residue quality and decomposition, future nutrient availability, and microbial populations). Our experiment directly measured only proximal impacts; nevertheless, nutrient cycling results from other research allow us to contemplate longer-term consequences. This research shows the importance of quantity (total amounts of C, N, and P in residue) and quality (C:N, C:P, lignin:N) on residence time in microbial nutrient pools, decomposition, and subsequent nutrient release (Cornwell et al., 2008; Ha et al., 2007; Mukuralinda et al., 2009; Yusuf et al., 2009)

Species adaptation and species-level attributes

Species showed a range of adaptation to site environment factors like soil type or temperature, which created large differences in biomass and associated attributes. Because sufficient biomass is vital for impacts of legumes via attributes such as P stocks or N fixation, it is useful to think of environmental adaptation in our experiment as a species-level filter. Environmental conditions varied across these fields, most notably soil texture, soil pH and temperature, and the legumes we evaluated varied in their response to these differences. For example, lupine was best adapted in light textured soils and mid to high elevations, vetch in heavy textured soils, and *arquilla* at low elevations in warmer temperatures. Endemic legumes seemed to be more precisely targeted in their environmental niches, while introduced legumes had high average performance with high unexplained variability, perhaps because they did not

tolerate low pH and/or drought at middle and high elevations. Interestingly, forage oat was widely adapted to these site factors, which explains its wide use by local farmers as a forage staple.

Differences in performance among legumes, and between legumes and oats, the benchmark grass species, illustrate the need for a species-specific approach to enhancing diversity using functional traits of legumes in agroecosystems (Drinkwater and Snapp, 2007; Eviner and Hawkes, 2008). For example, vetch was the best legume species for soil coverage by midseason, likely by virtue of a prostrate viny growth habit. *Arquilla* showed high %Ndfa at low-elevation sites where it was best adapted, but lower total Ndfa and biomass than other legumes, and lupine stood out as a non-mycorrhizal legume with high productivity and N fixation at fertile, low soil pH sites. Meanwhile, legumes as a group allocated a greater proportion of P below ground, and had narrower residue C:P and C:N ratios of both roots and shoots than oats. Forage oats accomplished high biomass and percent cover, and fixed C contribution to residues because of high P use efficiency. Species' attributes thus inform choices for legume intensification in these smallholder agroecosystems.

Soil fertility and feedback effects for degradation and restoration of agroecosystems

Although nutrient cycling attributes varied with plant species, soil P fertility strongly modulated these attributes, regardless of species, and would likely contribute to feedbacks for soil degradation or restoration. Phosphorus fertility changed attributes as varied as AM colonization, biomass N stocks, soil cover, and residue C:P ratios. Fertility thus had proximal or immediate impacts on plant attributes, which we would expect to cause future impacts on nutrition of subsequent crops, plant-microbial symbioses, and soil loss through erosion. These future impacts would likely strengthen feedbacks for degradation or restoration of soil nutrient cycling. Indeed our results suggest that low soil P fertility simultaneously reduces percent N fixed, total amount

of N fixed, soil coverage, and P stocks in residues, as well as fostering wider C:P ratios of residues. Low P fertility would thus foster mutually reinforcing conditions for reducing future productivity and soil fertility, accelerating degradation in the absence of new P inputs. Elsewhere we show that low soil P fertility in fields far from communities likely results from lower P inputs and greater soil erosion in these fields, establishing infield/outfield gradients (Vanek, Chapter 3). Here we conclude that legume attributes further reinforce these infield/outfield fertility differences.

The idea that degradation can be a self-accelerating process when thresholds are crossed via agroecosystem management has been explored by other researchers and is extended in useful ways by our experiment. Lawrence (2007) showed P fertility mechanisms by which shortened fallows in tropical dry forests could catastrophically alter nutrient cycling and accelerate degradation. Modeling approaches have shown how management feedbacks from crop failure and land abandonment likely worsened the dust bowl of the Great Plains in the 1930s (Cook et al., 2009). Much research has also elucidated feedbacks caused by overgrazing of arid shrub and grasslands that create thresholds and persistence of degraded states (Prober et al., 2002; Rietkerk, 1998; Schlesinger et al., 1990). Feedbacks between management and environment that restore soil fertility and enhance nutrient cycling have also been described: Peterson and Westfall (2004) describe positive feedbacks from increased SOM on productivity back to SOM when wheat-fallow rotations change to continuous no-till in the Western Great Plains, and Kimetu et al. (2008) tested how organic matter of differing quality could reverse degradation along a chronosequence in Kenya. Feedbacks that restore soil fertility are an implicit goal of those advocating deployment of functional diversity for improvement of agroecosystem function (Drinkwater and Snapp, 2007). Our research provides a blueprint for understanding this functional diversity and suggesting how feedbacks operate in smallholder

agriculture, across a number of attributes related to nutrient cycling rather than only those most closely related to productivity.

In our experiment, P-infertile sites seemed to fall below a degradation threshold, and legumes at these sites could not fulfill the expected benefits ascribed to legumes when replacing a grass species like forage oats. As an example we may consider N stocks by species in P-infertile fields (Fig. 2.2b). N stocks of only one legume – vetch – were moderately greater compared to oat. Total P in residues did not differ between legumes and oats for low fertility fields as we had expected, and C:P ratios of legume residues at low fertility might compromise subsequent P availability. At degraded, low P-fertility sites, P addition in organic or inorganic form would likely be effective in strengthening the functional role of legume attributes.

Soil N:P stoichiometry and legume symbioses

Soil P fertility and N:P ratios were also drivers of legume-rhizobia and legume-AM symbioses. Results on proportion and amount N fixed were consistent with other results showing that P limitation reduces N fixation at low soil fertility. Meanwhile, there was no suppression of BNF by high soil N levels and preferential soil uptake of N, even at more fertile sites. These results thus support the concept, drawn from theories of nutrient limitation and functional equilibrium, that proportion N fixed follows a 'hump' shape curve when plotted versus soil fertility. Further, BNF in these late-rotation, smallholder farm niches is located on the P-limited, ascending region rather than the N-saturated descending region of the curve.

With regards to plant-AM symbioses, our results display patterns predicted by the trade balance model: increased rates of colonization at higher soil $N:P_{inorg}$ and higher levels of biomass N:P are consistent with more mutualistic interactions between plants and AM fungi. Three-way coupling of soil N:P, biomass N:P, and intensity of AM symbioses suggests a fascinating positive feedback. By increasing the aggregate

residue N:P in soils via N fixation and fostering greater rates of colonization and production of AM propagules, mycorrhizal legumes could drive feedback effects that maintain or increase the success and mutualism of AM symbioses with host plants across the rotation. Research to analyze AM symbioses in longer-term rotations would be needed to confirm this hypothesis.

In an interesting contrast to mycorrhizal legumes, nonmycorrhizal lupine was the most responsive legume to higher P fertility for proportion and amount N fixed. Lupine's dramatic BNF response to soil P levels might have been due to its ability to directly partition fixed carbon to bradyrhizobia in the absence of a mycorrhizal symbiosis, which seems to have tended toward parasitism for the mycorrhizal legumes at higher soil P (low N:P) levels. Of course, poor tolerance of acidic soil pH of mycorrhizal pea and vetch is an alternate or additional explanation for this result suggested above. Comparing the response of BNF to soil P in mycorrhizal and non-mycorrhizal legume species is an interesting question for more reductionist research in agroecosystems.

Conclusions: matching plant species function with agroecological goals

At a practical level, our experiment tested ways that expanding species diversity with legumes in a late-rotation niche would increase nutrient availability to support food security in smallholder cropping systems. To achieve this goal, species specificity of legumes to environment should be recognized as a general result for smallholder systems in heterogeneous mountain environments, especially along soil texture, climate, and soil pH lines. This was exemplified by endemic legumes in our experiment, showing how species and varieties were adapted to particular elevation (temperature) niches. Vetch and forage pea's muted performance in low pH sites raised the issue of pH adaptation. Because soil liming is inaccessible to most

smallholders, finding legume cultivars that have robust productivity at a range of moderately acid soils (between pH 4.7 and 6.5) is important for these farmers in the highland tropics.

We also conclude that legume/forage grass mixes such as vetch/oats promise benefits for both forage supplies and soils. Oats was widely adapted in the study area and often had more favorable performance for biomass-based attributes other than N fixation (P stocks, percent cover, C additions to residue pools). Grass-legume mixtures might encourage adoption of legumes for mixed forage/green manure use, since enriching a popular forage crop by adding a legume greatly increases forage quality and requires only seeding another species rather than allocating additional land to legumes. One collaborating farmer also experimented successfully a lupine/vetch intercrop that was harvested to give forage, pulse, and soil fertility benefits. Legume-grass mixes thus use the advantages of both the benchmark species and the legume innovation, and seem a likely path towards optimizing functions of biomass nutrient stocks, soil coverage, forage supplies and forage quality, and residue quality for promoting P and N availability and longer-term maintenance of SOM stocks. Heinrichs and Aita show that mixture seeding rates can be tuned to optimize residue quality from vetch-oat mixes in larger-scale Brazilian agroecosystems (2001).

The importance of soil P fertility in modulating species attributes and degradation feedbacks is another main finding of our experiment. Modulation of legume attributes by soil fertility would likely apply to other cropping systems where gradients of soil fertility produce degradation, and where nutrient cycling relies on crop residues and organic matter inputs. Phosphorus additions to improve poor performance of legumes in P-infertile fields would help to reverse degrading tendencies, regardless of legume species. Our results also indicate that changes are

needed, however gradual, in harvest practices and ability of farmers to 'feed the soil' within the constraints of urgent subsistence demands for food and forage. A larger proportion of carbon and nutrients in the experiment was in aboveground biomass fractions usually harvested by farmers (a small proportion of farmers does in fact use green manures). If current harvest practices continue, the effects of deploying legumes or legume/grass mixes in the rotational niche we targeted would be limited to those attributes we identified for root biomass, such as root residue quality and AM colonization differences between oats and legumes. Short of farmers' dramatically expanding their use green manures, other changes to management could be attempted. Vetch and vetch/oats mixtures might lend themselves to this purpose, with early cutting of forage followed by regrowth to cover soil at season's end, or partial harvest in strips with incorporation or retention for soil cover of other biomass. Making farmers aware of root and shoot biomass pools could be used as an entry point to strategize with them about innovations that combine additional P sources, changes in harvest methods, and 'partial' green manuring.

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CHAPTER 3

MANAGEMENT, EROSION AND RANGELAND NPP IMPACTS ON SOIL NUTRIENT MASS BALANCES IN AN EXTENSIVE ANDEAN CROPPING SYSTEM

Abstract

In Andean potato-maize-cereal rotations, we tested factors affecting sustainability of soil nutrient stocks in smallholder mountain agriculture, using nutrient balances for (N), phosphorus (P), and potassium (K). We evaluated the role of socioeconomic indicators, elevation, distance from the community in an infield/outfield scheme, and MODIS remotely-sensed net primary productivity (NPP) of surrounding rangeland in determining balance outcomes for 43 fields in highland Bolivia. Anonymous community ranking by farmers of one another reflected wealth in terms of animal and land tenure, and wealthier farmers applied greater total amounts of manure nutrients per year. However, per-hectare manure nutrient application rates were not predicted by social rank but rather by infield/outfield typology and rangeland NPP. Crop nutrient exports over three years of rotation showed a pattern of progressive nutrient depletion after manure applied in the first year. Crop P exports were strongly related to the infield/outfield typology, with outfields showing lower exports. Crop and manure nutrient contents were less variable than were total inputs and exports. Harvest exports were proportional to manure nutrient inputs, so that net balances were less variable than either term of the balance. Calculated without erosion, N and P balances were positive. With erosion, N and P balances in far fields were negative, while near fields' balances were not different from zero. K balances were negative with or without erosion. The work shows that P is likely a limiting nutrient, with greater P deficits occurring further from communities. Erosion rates for any given field were a strong determinant of nutrient cycling sustainability. Results raise concerns about

negative impacts of net K exports over the long term. Balances for several scenarios reflecting changes from *status quo* management show the potential of P addition and erosion reduction to dramatically improve long-term nutrient trends in far fields.

Introduction

Evaluating the sustainability of agricultural productivity in rural smallholder agroecosystems is an important role for ecosystem and soil science. Soil nutrient management by smallholder farmers is characterized by diversity of approaches and outcomes for the agricultural landscapes they inhabit. Traditional methods used by low-intensity smallholders have sometimes been characterized as sustainable, relying on measured use of primary productivity in grazing areas and transformation of soil nutrients into useable form by fallow vegetation and animals (Pestalozzi, 2000). However, there is abundant evidence that these traditional systems are under stress and also create degrading tendencies through a variety of mechanisms such as market linkages (Pacheco, 2009; Pendleton and Howe, 2002), breakdown of communal management systems, and increasing human and animal populations (Baijukya et al., 2005; Lightfoot and Noble, 2001). In extensive agriculture utilizing fallows, these underlying causes may all be expressed in shortened fallow lengths that reduce potential of soil to regenerate soil fertility and increase rates of soil erosion by increasing the fraction of land in crops. Shortened fallows raise the concern that degradation will lead to a downward spiral of lowered productivity that in turn leads to hastened degradation. Eventually degradation creates a low-level equilibrium of production, which forms part of a panorama of poor food security of smallholders. However Scherr (2000) in fact used the term ‘downward spiral’ to focus criticism on it as overly simplistic, and others have critiqued the dire predictions of soil mining and soil degradation by smallholders as insufficiently cognizant of the creative responses

of farmers and less disastrous case-by-case analyses (Boesen and Friis-Hansen, 2001; Mortimore and Harris, 2005). In extensive agroecosystems, then, whether degradation is occurring is an open question, and research should critically address in detail the factors associated with sustainability of farmer management, in order to foster sustainable patterns of intensification.

Among these factors, our research sought to understand the degree to which wealth levels of farmers, productivity of rangelands that supply manure nutrients, and management affect soil fertility sustainability in an extensive smallholder agroecosystem. Simple input-output nutrient mass balances at the farm and field scale are well suited to this purpose. They are best used to reveal positive or negative trends in soil nutrients that result from the difference between nutrient additions and nutrients removed through environmental loss pathways or crop harvests (Smaling et al., 1996). Balances have been used in both industrialized and developing-country contexts, to quantify nutrients that may represent particular constraints in soils due to negative balances, or processes such as low inputs, crop export , or erosion that represent fulcrum points for changes in practices (Baijukya et al., 2005; Berry et al., 2003). Balances have also been used to identify which rotations, types of land use or geographic areas are particularly vulnerable to nutrient depletion (Elias et al., 1998; Lesschen et al., 2007; Wortmann and Kaizzi, 1998). Differences in nutrient balances with socioeconomic levels can gauge whether asset levels of farmers determine sustainability of agricultural practices, the concern being that either high or low wealth levels can drive degradation, because of market intensification or economic marginalization respectively (Cobo et al., 2009; Elias et al., 1998; Nkonya et al., 2005; Yirga and Hassan, 2006). In practice, studies often use nutrient balances to test several of these factors and questions simultaneously.

Research Questions:

In extensive, mixed livestock and cropping systems of smallholders in the Bolivian Andes, we used nutrient balances to evaluate the relative strength of management, social, and environmental drivers on the sustainability of soil fertility. The manuring rate of near and far fields was examined as a management driver that we hypothesized would create gradients in soil fertility. We considered land and animals owned by farmers as the most likely social driver of nutrient balances, since animal grazing generates manure which is virtually the only nutrient input. Likewise, rangeland NPP and erosion rates were likely environmental drivers of nutrient balances. These three types of drivers differ in causal proximity to the immediate decisions of farmers in crop production: manuring practices may create swift nutrient balance responses in the form of crop yield response to fertility, while environmental drivers such as erosion and rangeland NPP respond more slowly to degradation and innovations to reverse it. Differing livestock and land tenure are also likely more static factors than the management decisions of farmers, and comparing the balances of farmers with differing wealth levels can help to understand whether problems of soil degradation arise from wealth differences or are shared among wealthier and poorer farmers.

In spite of more and less direct coupling of these drivers to farmer practices, we hypothesized that these management, social, and environmental factors would all have impacts on the results of nutrient balances. First, we expected that farmers with more animals might apply larger amounts of manure nutrients, though realizing that this might also be determined by land tenure, so that reduced per-area rates of manure might occur in farms with larger landholdings. We also predicted that greater NPP for a community would translate to differences in inputs to agricultural fields, since rangeland productivity and crop residues are the primary source of animal manure.

We expected that fields nearer to communities with shorter fallow lengths would receive greater amounts of manure nutrients and also have more positive balances. In addition, we expected that soil erosion would have a strong negative impact on nutrient balances because of sloped topography in a mountain agroecosystem.

These questions also address management-related questions applicable to other extensive agroecosystems. By comparing manure inputs, crop export, and soil erosion as drivers of nutrient balances in near and far fields, we identified which of these nutrient flows constrains productivity over the long term and which type of field might be most impacted by changes in soil fertility or soil conservation practices. Nutrient balances also tested relative nutrient limitation by the three crop nutrients nitrogen (N), phosphorus (P), and potassium (K), which has implications for crop productivity and processes such as N fixation.

To illustrate comparisons among input and export flows and demonstrate likely impacts on soil fertility of alternate management, we developed several scenarios to test impacts of future changes in management. Scenarios compared different types of intensification to a *status quo* rotation based on balances we measured on farmer fields, namely: further intensification of agriculture using only shorter fallow lengths; shortened fallows combined with P addition and more intensive use of legumes; and combined P addition, legume use, and explicit measures to reduce erosion. Erosion control measures would include live barriers of perennial forages and double depth furrows for soil erosion capture, which are both in use by some farmers in the area.

Materials and Methods

Research area

We evaluated nutrient balances in five communities of northern Potosí, Bolivia, a region characterized by relative isolation from markets, potato-maize-cereal

rotations, and extensive management of crop fields set within large areas of rangeland and fallows. Elevations range from 2700 to 4000 meters and average rainfall is 650mm, occurring in a rainy season from October to March (FAO, 2010). Mean growing season temperatures range from 9.5ºC to 18.0ºC depending on elevation. Soils are dominantly eutric leptosols with some eutric and dystric cambisols in fields with deeper soils (Dijkshoorn et al., 2005). Agricultural fields follow an infield/outfield scheme, with fields in the immediate vicinity of the community under more intensive rotations than those far from the community. Fields far from the community generally formed part of a sectoral fallowing scheme where privately owned plots are managed as a block of fields with similar, synchronized crop rotations and fallows (Pestalozzi, 2000). Fields near the community are managed at a household level, with unsynchronized rotations and shorter fallow lengths. Rotations in most fields begin with potato as the first crop in conjunction with manure addition, followed by a second year with maize, wheat, barley, fava beans, peas, oca or other minor Andean tubers. A final rotation year follows, usually with forage oats which is a major forage staple. Andean lupine (*tarwi* locally, *Lupinus mutabilis* Sweet) occasionally occupies the final year of the rotation. Legumes occur about once per three rotation cycles (25y) in any given outfield and once per seven years in infields (Jones and Vanek, unpublished data, survey 2009). Maize is grown only below 3600m elevation, and is sometimes intensively cropped on infields at low elevation in continuous rotation alternating with wheat.

Aside from legume N fixation, manure inputs harvested from rangeland are generally the only fertility input to fields. Manure is applied in two ways. In communities at low elevation (2500-3400 masl), animals are corralled on fields in the year prior to cropping. Each area of a field receives two to four nights of manuring, with potato receiving more nights than maize, while wheat generally receives only

sporadic manure inputs. A second form of manure application is used throughout the elevation range, and is the only method used in communities above about 3500 m: manure is gathered year-round by bedding animals in fixed pens and then carried using pack animals to fields as part of secondary tillage and potato seeding.

Wealth ranking and land and animal assets of farmers

Data on household asset levels was provided to us by World Neighbors from rural appraisal focus group activities as part of a baseline assessment in August 2005. Data included anonymous wealth ranking carried out by groups of community stakeholders into three groups: 'those with most'; 'those with less'; and 'those with very little', as well as defining characteristics of different asset levels (approximate number of animals, amount of major crop types seeded). Farmers whose fields we sampled for nutrient balances were asked the number of animals they owned and amounts of seed in a typical year for each staple and minor crop they grew (in customary units, then converted to kg). Seed amounts were used to calculate total cropped area belonging to the household, dividing amount seeded by mean crop seeding rates in $kg \cdot ha^{-1}$ agreed upon with agronomists with experience in the area. We opted for this approach rather than direct questions to farmers about land area because seed amounts were felt to be more precise than area estimates of fields. Also community leaders and agronomists advised that farmers were sensitive to direct questions about landholdings.

Field selection and overall balance approach

Fields for measurement were selected by requesting permission of farmers to gather samples in fields with their help or the help of a farmer field worker, based on random selection from a listing of community households, or advice of a farmer field worker if first selections were not available. Selections were stratified by anonymous wealth ranking, and we succeeded in gathering information from all three wealth

levels in all communities. However, the middle wealth group was always the largest in any communities' ranking roster, and the final sample reflected this with only 10 each of highest and lowest grouped-farmers, and 23 of the middle group for 43 fields total.

Balances were calculated as of inputs minus outputs for an entire rotation, divided by the length of the rotation. Rotations were two years long for infields in the lowest community, where near-continuous cropping of maize and wheat takes place, and six years long for most other fields. Some infields at middle and high elevations had three-year, repeating rotations with continuous cropping. Because the length of the fallow previous to or following the measured cropped period was not asked of farmers, we used three years as the fallow length for far fields based on projections of likely fallow length by farmers and the median fallow length (3.2 y) from a parallel nutrition survey of 330 households (Jones and Vanek, unpublished data).

Because of time constraints on fieldwork, we were able to directly measure sequences of manuring and two initial crop years on the core sample set of 43 fields, during which the largest nutrient inputs and exports occur. To estimate yields in these fields for a third and subsequent year occupied by cereal crops, we sampled a separate set of fields in the third year of the rotation. These fields were in the same communities, infield/outfield locations, and elevations as those sampled for two-year sequences. We then fit the samples from the third-year fields to a random normal distribution to generate random P export quantities for the third year, and found N and K exports for these fields by multiplying by a similar random draw of the N:P and K:P ratio of crop exports for this set of third year fields. When lupine was grown in the third year, yields were estimated by asking the farmer for a qualitative estimate of yields and converting this to amounts of N, P, and K using experimental data for nutrient uptake of lupine at flowering (Vanek, Chapter 2).

MODIS-derived NPP within community grazing and farming areas

Remotely sensed net primary productivity (NPP) for the research area was downloaded from http://modis.gsfc.nasa.gov/ or the equivalent previous MODIS site in April 2009 (file *Npp_1km_C5.1_mean_00_to_06.tif*). This file gives the average 2000-2006 value for annual NPP at a 1 km^2 grid size. A georeferenced topographic map and digital elevation model was then used to mark community boundaries designated by collaborating farmers, and extract an average value of annual NPP for the 5 to 10 1-km^2 pixels covering the community.

Manure inputs to fields

Measurement of manure inputs of N, P, and K reflected the two systems in use for manure application. Our methods are most accurate for P inputs, and entail uncertainty in the estimates of N and K for the system of corralling on fields at lower elevations. In two communities where this system was used, manure was gathered in three quadrats of 0.25 m^2 within the corral. The corral was stratified into three sections, with one randomly located quadrat per section. In the case that manure on the soil had created a moist pack, care was taken to include a small amount of adhering soil so as to capture the entire solid mass of feces. Each quadrat's manure was weighed and subsampled for drying and nutrient analysis (below). Dry weight of manure from each quadrat was divided by 0.25 m^2 to calculate an application rate and averaged across three quadrats for a field-level nutrient application rate. Manure rates were measured after at least two nights of manuring. We then multiplied a per-night rate from these measurements by the total number of nights the farmer intended to manure the field (usually three or four nights).

For corralling on-field, we did not account for urine N that entered the soil below the layer of manure deposited on the soil surface. The magnitude of this

omission would likely depend on forage diet quality and the fate of urine N entering the soil. A transect of forage grab samples in rangeland at the end of the rainy season revealed a likely overall diet N content of between 1.5 and 2.0%, which means that N amounts in urine may have approached those in the manure organic solids, causing non-negligible errors in our measurements (Barrow, 1998; White et al., 1997). However, gaseous loss of urine N before incorporation to soil stocks may have obviated this error, since conditions at the end of the rainy season make it likely that 50% or more of urine N may have been lost to volatilization (Powell et al., 1998). Urine near the soil surface was subjected to dry, hot days, with manure left on the surface for several days, followed by tillage, an intense dry season, and further tillage for seeding, conditions conducive to volatilization of ammonium N derived from urine. On the other hand, nitrification, sorption of ammonium on soil cation exchange sites, and microbe assimilation to organic N forms are also possible fates of urine N in aerated, drying soils at the end of a rainy season (Williams and Haynes, 2000), counteracting losses to volatilization. We estimated that missing urine N inputs might represent a potential error of up to 20% for fields where livestock was corralled on fields.

The same problems of omission might also apply to potassium, which is exclusively excreted via urine by ruminants when in surplus in the diet (NRC, 2007). Potassium contents of corral-based manures, where urine K may have entered the soil, were indeed less than those transported from fixed household manure packs, accentuating this concern. We therefore multiplied K contents of manure from moveable corrals by 1.96, a correction factor based on one middle elevation community where several applications of each type were measured, assuming that the differences in K content between pack and moveable corral manure represents missing K lost from the layer of manure deposited by animals over several nights.

The second system of manure application, using dry pack manure from household pens, was measured immediately before planting in September and October and had fewer possibilities for error. Nitrogen and K errors when measuring dry pack manure applications were negligible because no liquid portion of the manure can move into the soil. Manure is transported to the field at moisture contents under 50% and rainfall is not heavy during the application period. Measurement of manuring rates in this system was facilitated by the fact that manure is piled in a regular way in the field in preparation for seeding. We could therefore measure rectangular areas, or the entire field, enclosing 15 to 20 manure piles, and then estimate the total weight of manure enclosed by the rectangle. Length and width of the rectangle was measured to calculate area. To estimate manure weight, we weighed two representative piles with a spring scale and bucket to calculate bulk density of the manure piles. Spring scales were calibrated with a known volume of water to reduce error. Total volume of manure in the weighed piles, and the remaining piles whose weight we estimated, was calculated by measuring height and major and minor diameter of all piles within the measurement area, and modeling the volume of piles as a cone with an elliptical base and a rounded top. We then used the manure bulk density to calculate the total mass of manure in all manure piles, summing to find the total mass of manure applied within the measured rectangle and then the manuring rate as total mass per measured area. Two handfuls of manure were gathered from the interior of each pile and bulked in a bucket, mixed, and a subsample taken for drying and nutrient analysis (below). Dry matter content was used with the total weight of piles and the measured area to calculate dry manure application rates.

Seed nutrient inputs

Seed nutrient inputs were calculated at standard rates for the area agreed upon with local practitioners and field staff, as follows: potatoes 1200 $kg \cdot ha^{-1}$, maize 80

kg·ha^{-1}, wheat and barley 100 kg·ha^{-1}, oats 120 kg·ha^{-1}, fava beans 120 kg·ha^{-1} and lupine 60 kg·ha^{-1}. These seed rates were multiplied by the nutrient contents of grains from our nutrient sampling. In the case of oat seed a literature value was used from a nutrient budgeting tool (NRCS, 2010). Seed nutrient inputs were small, the largest being K for potatoes with 5.6 kg K·ha^{-1}.

N fixation

N fixed by legume crops (fava beans and lupine) was estimated as a net zero input or export, based on our experiments with lupine in the area. These results showed that the above-ground fraction of N in lupine, 69% (versus taproots, fine roots, and nodules) was approximately equal to the proportion of N fixed, so that crop N exports equaled the amount N fixed for a net zero contribution to field N stocks. This is a conservative assumption given other estimates by Ross et al. (2008) of N fixed by fava bean (75%) which matches rates we measured in the most P-fertile sites by tarwi in the area (Vanek, paper 2, 80%), and also results of Villaroel et al. (1986) that Andean lupine provided about 16 kg·ha^{-1} residual N in a P-fertile soil. Net N inputs by pulse legumes were thus plausible. However their contribution in any case would remain small on an annualized basis because of infrequency of legumes in the rotation.

In sampled fields where three-year fallows occurred, these were also assigned a net N input value due to N fixation of 12 kg·ha^{-1}. This was based on sampling of three fallow fields of age two to five years, with mean total N stock in legume tops and taproots of 24 kg·ha^{-1}. We then used an estimated mean %Ndfa for several endemic wild legumes such as *Trifolium amabile* and *Parocela pacense* of 50%. This figure does not count fixed N from legumes that may have been cycled back to the soil by manure deposited during grazing of animals in the fallow period. Manure deposition is potentially a non-negligible additional flow since grazing animals return about half of their diet back to rangeland when grazing. If we estimate the additional

N fixed and returned to rangeland as equal to the estimated N fixed in fallows (12 $kg \cdot ha^{-1}$ over three years of fallow), N fixed in rangelands would be underestimated by two $kg \cdot ha^{-1}$ per year across a six year rotation with three years of fallow. Since this correction is small, and very approximate, we omitted it from the balance.

Crop exports

Crops were sampled in a way that best approximated farmer practice in the export of crops and residues from the field. In every case, three stratified random samples of yield were taken in different parts of the field. Stratification was sometimes used to sample separately parts of the field with different slopes or other characteristics. Crops and residues from small areas (2-3 m^2) were weighed and the mass divided by the area of each quadrat to give a yield in $kg \cdot ha^{-1}$. Field yield was calculated as the average of yield or biomass for the three quadrats.

Potatoes and oca (*Oxalis tuberosum*) harvest samples were dug in three areas of 2 m by two rows at three locations in field. Inter-row distance was measured to calculate harvested area of each quadrat. Total fresh harvest mass of potatoes was weighed with a digital scale, using the guidance of farmers to discard potatoes that would normally be left in the field. In practice almost all potatoes were taken, because damaged tubers are fed to animals. From each area, a sub-sample composed of one small, one large, and two medium potatoes was taken, washed and reweighed to correct for soil weight in the fresh sample. A representative sample of thin slices from these potatoes was weighed and frozen before drying and nutrient analysis (below).

Maize was harvested after physiological maturity (when the black layer forms between kernels and cob) in three areas of two rows by 2m, before farmer harvest. Total fresh plant biomass and total weight of ears with grain from the area was weighed. Two representative ears were weighed as a subsample, dried, and shelled as a subsample for nutrient analysis (below). The grain subsample was weighed to give

the mass of dry grain per fresh ear mass at sampling, to calculate dry grain from the total harvested ear mass for each quadrat. Stover biomass was calculated using a subsample of two representative whole plants after removing ears. These plants were chopped into segments, each second segment discarded, and the remainder bulked as a subsample. Stems of large plants were crushed to facilitate drying. Dry matter content was multiplied by the stalk and leaf biomass at harvest, and added to cob and husk biomass to calculate stover biomass for each quadrat.

Cereal crops (forage oats, wheat, and barley) were harvested in three 1-m^2 quadrats per field. The entire mass of stems, leaves and grain was weighed. Twenty-five random stems with leaves (every three stems until 25 were taken) were taken as a subsample. For forage oats, this entire sample was dried and weighed to calculate dry matter content and yield. For wheat and barley, the sample was first hand-threshed to separate between grain and a fraction representing chaff plus straw (residue). Each of these was dried and weighed to calculate the proportions of dry grain and residue resulting from fresh biomass in the quadrat at sampling. These ratios were multiplied by the entire harvested sample mass to find grain and residue yield for each quadrat. Farmers remove residue with grain for threshing in a central location followed by feeding to livestock, so we treated residue as an export. We cut our samples at a few cm above the ground to replicate the effect of any subsequent animal grazing on stubble. In addition, in some fertile fields considerable weed biomass was grazed after harvest, and so weed biomass was also sampled and added to the export nutrients.

Moisture content and nutrient analyses

Plant samples were dried inside bags on a rooftop in strong sunlight (45ºC), followed by oven-drying at 58 ºC to constant weight. Manure was air-dried in bowls in a dry greenhouse. Dry matter content was determined as the dry weight divided by the subsample fresh weight taken in the field. After drying and grinding to 30-mesh

size, total N content was measured in crop, residue, and manure by combustion (LECO C and N analyzer, St. Joseph, MI). Total P and K in dry biomass fractions was determined using a nitric acid digestion of ground plant tissue with H_2O_2 addition to oxidize organic carbon in the final digestion steps (Kalra, 1998). National Institute of Standards and Technology apple leaf standards were used to verify >92% recovery of P from samples. Digested residue was dissolved in 4% HCl and analyzed for K and P using inductively coupled plasma atomic emission spectrometry (Isaac and Johnson, 1998). N, P and K stocks in crop, residues, and manure were calculated as:

C, P, or K stock ($kg \cdot ha^{-1}$) = biomass of fraction ($kg \cdot ha^{-1}$) * % nutrient (1)

Erosion losses

To estimate erosion for the measured fields, we performed a local calibration of the Revised Universal Soil Loss Equation (RUSLE) over one growing season on six fields with cereal crops and six fields in fallow (Renard et al., 1997). Measured erosion rates were regressed to the LS topographic factor representing slope length and gradient from the RUSLE equation for measured fields ($E= R \cdot K \cdot L \cdot S \cdot C \cdot P$). In this way erosion rates in fields with crop and manure measurements could be estimated using $E_{modeled} = M_{local} \cdot LS$, where M_{local} was the fitted slope of the measured erosion rates against LS, effectively including all other constants in the RUSLE equation [$M_{local} = (R \cdot K \cdot C \cdot P)$] For calibration measurements, we modified the erosion pins method (Haigh, 1977; Hudson, 1993) in which denudation or deposition is measured as change in elevation of the soil surface compared to a fixed reference point. On each field, we installed four sets of triangular arrangements of stakes. Triangles were approximately equilateral with side 60 to 80 cm when viewed from above. Stakes were 80 cm long, made from 9-mm diameter concrete reinforcement rod, and driven into the ground so that 10 to 15 cm protruded. A notch was made at the top of each stake with an angle grinder to serve as a reference point or datum for winding 3-mm

cotton string into a triangular shape suspended above the soil at a height of about 10 cm. The string was marked every 10cm with permanent marker so that a height to the soil surface could be taken with between 15 and 20 replicates at each set of stakes. Care was taken not to disturb the soil surface within the perimeter or edge of the triangle during installation, and an initial set of heights from each point on the string were taken in millimeters after installing the stakes. Measurements were repeated at the end of the rainy season after harvest. The string was wound in the same configuration on the triangle, and with the same tension at both initial and final measurements by noting the distance between the last mark on the string and the final winding on a stake. In this way the same vicinity of the soil surface (+/- 1 cm) was being measured at initial and final measurements. The same string and millimeter ruler was used for both initial and final measurements.

Change in elevation of the soil surface was taken by averaging the differences between final and initial height for each point on the string (average elevation change = Δh). At both initial and final measurements of soil height, we measured bulk density of the soil ($r_{b\text{-}i,}$ $r_{b\text{-}f}$) to correct for the elevation change in the soil associated with settling and compaction from the total lowering measured. Five undisturbed soil cores of known volume were taken to 20 cm, combined, and weighed for moist density. Cores were taken within 1m of the stakes but not immediately next to them to avoid impacts on erosion or deposition of soil. To determine dry bulk density (r_b), gravimetric moisture was determined on bulked soil samples by drying at 105ºC to constant weight. Stones of diameter >2mm were also weighed after sieving dried samples, to calculate soil content of small stones. We then determined the ratio of final to initial r_b, correcting for stone content differences between final and initial sampling. We also corrected for lowering of the soil surface and inclusion of deeper

(thus denser) soil by cores of 20cm depth, in accordance with the principles of elevation-based soil sampling (Chang et al., 2007).

Calculations to correct for differences in stone content between initial and final sampling were as follows: differences in measured soil bulk density can arise due to higher or lower proportion of stones in a given sample, and the higher bulk density of rock then soil, rather than actual compaction and shortening of the soil profile. We thus standardized the final bulk density to the stone content of the initial soil sample ($r_{b\text{-}f,\ std}$), using dry weights and bulk densities:

$$r_{b\text{-}f,\ std} = [m_{soil\text{-}f} + (\Delta m_{rock} * r_{b\text{-}f,\ soil}/\ 2.5) + m_{rock\text{-}i}] / V_{tot\text{-}\ f} \qquad (2)$$

Where $m_{soil\text{-}f}$ is the mass of soil only in the final sample for bulk density; $\Delta m_{rock} = m_{rock\text{-}f} - m_{rock\text{-}i}$ or the difference between final and initial mass of rock in the samples; $r_{b\text{-}f,\ soil}$ is the bulk density of just the soil without rocks in the final sampling; 2.5 is an estimate of stone bulk density; $m_{rock\text{-}i}$ is the mass of rock in the initial sampling; and $V_{tot\text{-}\ f}$ is the final total volume of the soil sample. If stone content is higher in the final sampling, this adjustment lowers $r_{b\text{-}f,\ std}$ slightly by replacing the difference in sampled stones with an equivalent volume of soil with the same r_b as at final sampling. When stone content is lower at final sampling, the correction augments $r_{b\text{-}f}$ in a corresponding way.

Calculations to correct for lowering of the soil surface and inclusion of deeper soils at the final sampling were as follows: we assumed a subsoil bulk density ($r_{b\text{-}subsoil}$) of 1.6, or higher when warranted by the surface r_b of the site measured in initial and final sampling. We then calculated final r_b corrected for soil lowering ($r_{b\text{-}f,corrected}$), essentially subtracting out the effect of denser soil layers below those sampled initially that were sampled at the end because the surface had been lowered:

$$r_{b\text{-}f,corrected} = [r_{b\text{-}f,\ std} - (\Delta h/20 * r_{b\text{-}subsoil})]/[(1- \Delta h/20] \qquad (3)$$

Where Δh is the amount of soil lowering in cm, the result of combined settling and erosion, and 20 is the soil sampling depth in cm and $r_{b\text{-}f,\ std}$ is taken from (2) above. We then found the ratio of final to initial bulk densities corrected for the same soil depth from the reference point provided by the erosion pins:

$$S = [r_{b\text{-}f,corrected}/r_{b\text{-}initial}] \quad (4)$$

We then used the following equation to correct the measured soil lowering Δh for changes due to compaction:

$$\Delta h_{\ corrected} = \Delta h - (20\text{-}20/S) \quad (5)$$

$\Delta h_{\ corrected}$ was then multiplied by $r_{b\text{-}avg}$, the mean of $r_{b\text{-}f}$ and $r_{b\text{-}i}$ to give erosion soil loss or deposition ($Mg \cdot ha^{-1}$) using standard conversion of units:

$$E\ (Mg/ha) = \Delta h_{\ corrected}\ (cm) * r_{b\text{-}avg}\ (Mg/m^3) / 100\ (cm/m) * 10000\ (m^2/ha) \quad (5)$$

Erosion or deposition rates from the four staked sites were averaged to provide an estimate of erosion or deposition for the field. For fallow fields, we did not adjust for density changes because we assumed constant soil r_b as is the case for the erosion pins method on non-agricultural sites, and Δh was used directly in eqn. (5) above.

To find N, P, and K lost to erosion, the erosion or deposition rate was multiplied by total N and P content of the soil and an estimate of soil labile K (two times the exchangeable K or K_{exch}). Nitrogen, P, and K_{exch} contents of soil were measured by combustion, perchloric acid digestion, and ammonium acetate K_{exch} respectively. Soil was bulked among field replicates for field-level estimates of erosion losses. Exchangeable K was used as a basis for K erosion losses because total soil K erosion flows would have dwarfed manure and crop export flows, rendering analyses meaningless. Also, manure and crop exports and manure are chemically more similar to K_{exch} than to total geochemical K pools. Enrichment factors for N and P in soil erosion losses have been used by other authors because these nutrients are associated with organic matter fractions that are eroded preferentially from soils

(Menzel, 1980). However we ignored enrichment factors since we did not install erosion plots been needed to measure them, and because enrichment factors are less important in high-erosion regimes, which we expected for these mountain agroecosystems.

For calculation of the RUSLE topographic or LS factor, slope length and slope angle was measured on fields where erosion was measured using stakes. Slope angle was measured using a 2m straightedge and spirit level. For fields where crops and yields was measured, slope and length were either measured directly or reconstructed using photos of the site and checked with an ASTER digital elevation model. The LS factor was calculated from Renard et al. (1997).

Estimation of leaching and gaseous losses

Nitrogen and K leaching and gaseous losses of N were estimated using transfer functions from the NUTMON approach (Lesschen et al., 2007) . These equations depend on amount N or K applied, annual rainfall, soil clay content and cation exchange capacity, and organic matter content and breakdown rate. We used area-wide estimates of these properties because we did not have detailed soils data on fields measured for nutrient balances. Even with detailed soils data, these N and K estimates likely represent upper bounds rather than because they were developed for lowland systems with greater rates of decomposition and soluble fertilizer use.

Scenarios of alternative management

Three scenarios of management alternatives to the *status quo* were developed for 18 rotation years on a typical model field far from a community. For erosion calculation based on RUSLE, the field had a 15 m slope length and 10% slope gradient. Cropping sequences and manuring rates are shown in Table 3.6. Mean N, P, and K inputs to sampled far fields from the balances were used as the manuring rate for years in the scenario rotation when potatoes were planted. Yields of all scenario

crops were drawn from the 95% confidence interval of a random normal distribution fit to the population of crop harvest data for each crop. We assumed that P addition to oats and vetch forage crops and tarwi green manures would decrease erosion by 10% by fostering greater soil cover, and that more aggressive actions to curb erosion could reduce by 50% the amount of erosion. The N, P, and K budgets for these rotations were composed in an Excel spreadsheet that we replicated 16 times with different random draws for crop yields. No statistical inferences were drawn from these scenarios, but we report the standard deviation as an indication of the variability caused by crop yields.

Statistical analyses

For data linking wealth ranking to land an animal tenure, analysis of variance (JMP, SAS Institute, Cary, NC) was used to test mean separation of different groups of farmers. Analysis of covariance was used to test categorical and continuous predictors of manure application rates, manure content, crop nutrient content, crop nutrient exports, and full balances for N, P, and K. Categorical predictors were year in the rotation, near/far status of fields, and wealth ranking of farmers, while community NPP was a continuous predictor. Community was first included as a random effect, but was dropped because it made no difference to the results of analysis.

Results

Manure inputs: economic, ecosystem NPP, and infield/outfield factors

Results for manure inputs showed several trends. Anonymous wealth ranking by farmer peers matched numbers of animals and land area reported by direct interviews of farmers (Table 3.1). By contrast, manure application rates for N and P were strikingly constant across economic levels, refuting our hypothesis that farmers would differ by wealth levels in their manuring rates. This is likely because cropped land area and number of animals increase proportionally: farmers with more land to

Table 3.1. Community social ranking as a predictor of livestock tenure, cropped and manured area, manuring rate, and total manure nutrients applied. Total N and P applied are calculated on a farm basis multiplying manure application rate by the reported area in maize and potatoes that are potential crops for manure application. Social ranking data were provided by World Neighbors as part of their 2005 project baseline on communities in a legumes project (Sanchez, 2005).

Community social ranking from RRA	number of animals	Total cropped land area, ha.	Land area in potatoes/ maize (for manuring)	mean manuring rate, kg·ha^{-1}·y^{-1}		Total nutrient applied per year, kg, estimated	
				N	P	N	P
'most'	72 a	1.83 a	0.60 a	130	32	52 a	12.0 a
'less'	56 a	1.30 b	0.46 a	119	28	47 a	10.9 a
'very little'	23 b	0.69 c	0.23 b	123	28	23 b	5.3 b
Significance	p<0.001 ***	p<0.001 ***	p<0.001 ***	ns	ns	p<0.01 **	p<0.01 **

manure also had more animals and therefore transferred more manure from rangeland. When inquiring about the poorest farmers, we learned that even farmers with no animals have access to manure through barter arrangements in which they tend wealthier farmers' flocks in exchange for manure. However, total manure applied by wealthy and middle-group farmers was indeed greater compared to the poorest farmers, reflecting differences in land area requiring manure of these three groups (Table 3.1). The poorest group had dramatically fewer animals and land area than the middle and wealthiest farmers, suggesting that agricultural productivity and food security for this group is the most precarious of the three.

Meanwhile, communities with higher NPP, driven by rangeland NPP as the largest proportion of land use, had higher application rates of N and P as well as manure N and P content (Fig. 3.1, Table 3.2). This supported our hypothesis that productivity of surrounding rangeland might be a driver for nutrient management by farmers. At the highest-NPP community, we observed larger manure piles on fields and greater amounts of tree and shrub browse for goats, including several important legume species. The lowest NPP community in Fig. 3.1 had the highest proportion of steep land (85% land with slope gradient >17%, data from digital elevation model) and visible rangeland degradation that likely contributed to low manure nutrient application rates and nutrient content.

Distance from the community had a large impact on N, P, and K inputs to fields. There were highly significant differences in N, P, and K application rates between fields located more than 500 m from the community (outfields) and those located near to communities (Table 3.5, top). Table 3.5 shows annualized trends for nutrients, which deflates manure application rates in far fields because the application rates are divided by a larger number of fallow years in far fields. However, even manure application rates considered in the year that manure was applied differed

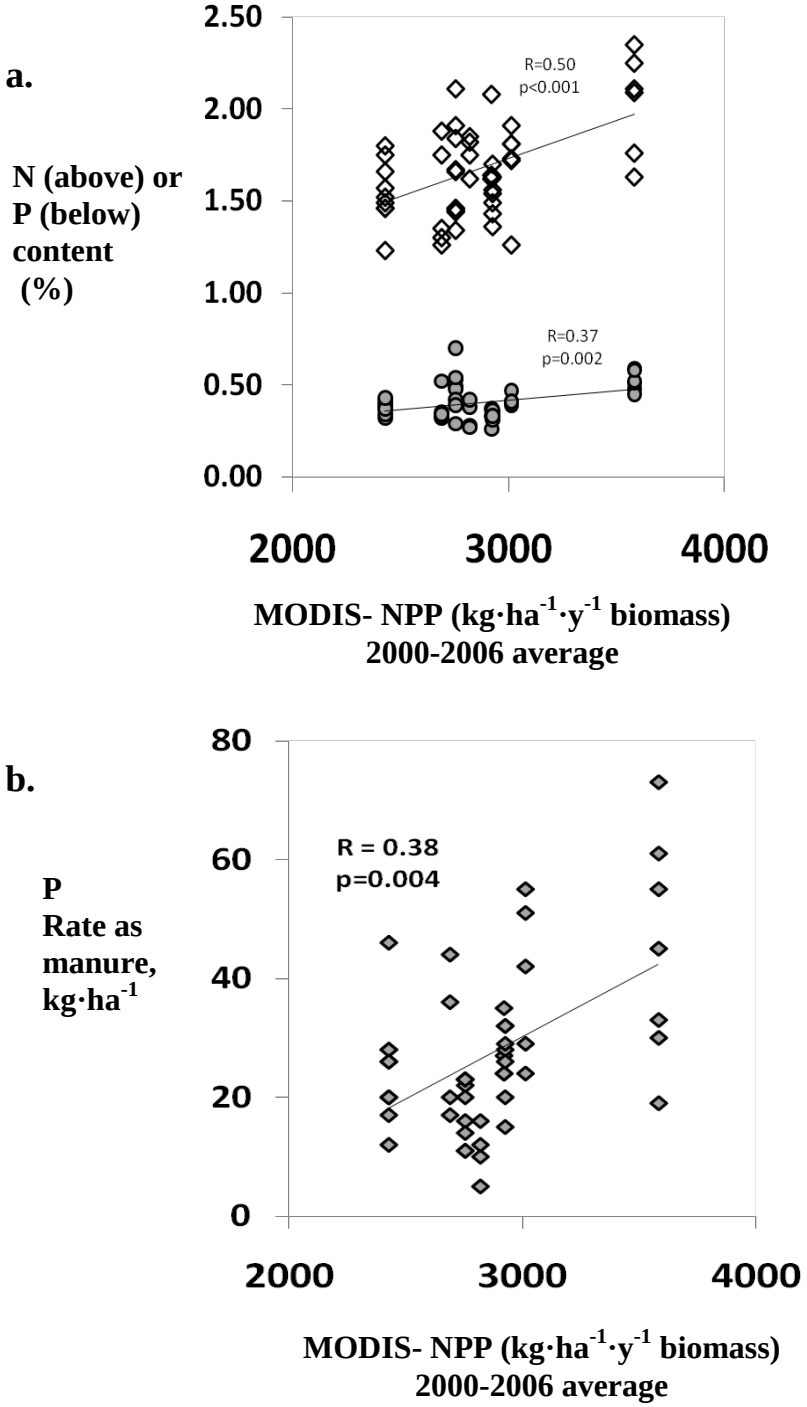

Figure 3.1. Dependence on remotely sensed NPP of a. manure N and P content and b. Phosphorus application rate in manure. NPP data is the MODIS-remote sensing derived average of 2000-2006 data for the area of a community.

Table 3.2. **a.** Regression slopes of nutrient content and nutrient application rate on remotely-sensed NPP for community (MODIS); **b**. Mean nutrient content of manure in two different manuring strategies. *p<0.05; **p<0.01; *** p<0.001.

a. regression coefficients of nutrient content and application rates to MODIS-derived NPP for 2000-2006

Nutrient in manure	**N**	**P**	**K**	
	coefficient and significance			**Units of coefficient**
Nutrient content of manure	0.41 ***	0.004**	1.26 ***	% of nutrient per $Mg \cdot ha^{-1}$ NPP
Nutrient application rate, (in application year)	63.8*	14.0 *	137 ***	$kg \cdot ha^{-1}$ per $Mg \cdot ha^{-1}$ NPP

b. Nutrient contents of manure for different manuring strategies

	N content (mg/kg)	**P content (mg/kg)**	**K content (mg/kg)**
Fixed household pen, manure carried	**16.9** ^{ns}	**3.8** ^{ns}	**16.9** *
	(0.4)	(0.4)	(0.8)
Animals corralled on field	**16.5** ^{ns}	**4.4** ^{ns}	**9.2** *
	(0.6)	(0.6)	(1.2)

significantly between near and far fields. For the case of P, application rates were 39 kg $P \cdot ha^{-1}$ in near fields and 24 kg $P \cdot ha^{-1}$ in far fields (F for comparison =13.8, $p<0.0001$).

In contrast to the impact of NPP and distance from the community, the two manuring systems did not differ in N and P content of manures (Table 3.2). They did differ in K content, a difference that persisted even though the manure K contents from manure in moveable corrals had been adjusted upward by almost a factor of two prior to analysis. In any case there was greater uncertainty in our measures of K in field balances, especially where corralling animals on the field was used to apply manure.

Crop exports: rotation year and near/far field impacts on nutrient content and nutrient exports

Crop nutrient contents and nutrient exports varied in ways that reflected the strong differences in nutrient inputs between near and far fields, and also suggested year by year depletion of nutrients and smaller nutrient export in years following application of manure (Tables 3.3 and 3.4). Trends in nutrient content were weaker than those for total nutrient export. For example, P and K content of maize grain were somewhat lower in the second year after manure than when manure was applied to these crops, but other crops showed no differences in nutrient content between different years of the rotation. Meanwhile, differences in nutrient content were notable between near and far fields, with higher N content of grain, P and K content of potatoes, and K content of cereal straw in near fields. In all but one case (N content of maize grain), nutrient content of crop and residue exports were higher in near than far fields, and when nutrient content was compared among near and far fields, averaging across crops, these near/far distinctions were significant for P and K content ($p<0.05$).

Table 3.3. Nutrient content for crops in different years of the rotation after manure application, and with respect to distance from the community.

	Year of rotation				*infield/outfield position*			
	one (manure applied)	**two**	**three**	*p value for difference*	**Near**	**Far**	*p value for difference*	*N (fields)*
Crop and fraction	**N content g·kg^{-1}**							
Potato	**9.8** (0.2)	--	--	--	**10.1** (0.4)	**9.8** (0.3)	ns	63
Maize grain	**13.1** (1.0)	**10.9** (0.9)	--	0.09 ns	**11.0** (0.7)	**13.1** (1.4)	ns	21
Maize stover	**3.9** (0.3)	**3.6** (0.2)	--	ns	**3.8** (0.2)	**3.7** (0.4)	ns	21
Cereals grain	--	**16.2** (0.4)	**16.6**(0.9)	ns	**17.7** (0.7)	**15.1** (0.5)	**0.003** **	23
Cereals straw	--	**2.9** (0.2)	**2.7** (0.3)	ns	**3.1** (0.3)	**2.5** (0.2)	0.08 ns	23
Forage oats	--	**4.5** (0.6)	**6.3** (0.8)	0.08 ns	**6.1** (1.0)	**4.8** (0.5)	ns	7
	P content g·kg^{-1}							
Potato	**1.7** (0.04)	--	--	--	**1.8** (0.1)	**1.6** (0.1)	**0.008** **	63
Maize grain	**3.7** (0.2)	**3.0** (0.2)	--	**0.002** **	**3.4** (0.1)	**3.2** (0.2)	ns	21
Maize stover	**1.6** (0.3)	**1.1** (0.2)	--	Ns	**1.6** (0.2)	**1.1** (0.4)	ns	21
Cereals grain	--	**3.5** (0.2)	**3.4** (0.4)	Ns	**3.5** (0.3)	**3.3** (0.2)	ns	23
Cereals straw	--	**0.7** (0.1)	**0.4** (0.1)	0.10 ns	**0.6** (0.2)	**0.4** (0.3)	ns	23
Forage oats	--	**1.8** (0.3)	**2.0** (0.4)	Ns	**2.5** (0.5)	**1.3** (0.3)	**0.04** *	7

Table 3.3 (continued).

	Year of rotation				*infield/outfield position*			
	one (manure applied)	**two**	**three**	*p value for difference*	**Near**	**Far**	*p value for difference*	*N (fields)*
Crop and fraction	**K content g·kg^{-1}**							
Potato	**18.0** (0.3)	--	--	--	**19.2** (0.4)	**17.4** (0.3)	0.008**	63
Maize grain	**6.5** (0.4)	**5.0** (0.3)	--	0.01**	**5.7** (0.3)	**5.6** (0.6)	ns	21
Maize stover	**14.8** (0.8)	**15.9** (0.6)	--	Ns	**15.9** (0.5)	**14.8** (0.9)	ns	21
Cereals grain	--	**4.9** (0.2)	**4.6** (0.4)	Ns	**4.9** (0.2)	**4.7** (0.3)	ns	23
Cereals straw	--	**9.5** (0.3)	**7.7** (0.5)	0.11 ns	**9.9** (0.5)	**7.4** (0.3)	0.02*	23
Forage oats	--	**14.9** (0.5)	**14.4** (0.8)	Ns	**16.6** (0.9)	**12.8** (0.5)	ns	7

Table 3.4. Yield and nutrient exports of crops according to year of rotation and location as infields and outfields.

	Year of rotation				*infield/outfield position*			
Crop and fraction	**one** (manure applied)	**two**	**three**	*p value for difference*	**Near**	**Far**	*p value for difference*	*N (fields)*
	Yield (Mg·ha^{-1})							
Potato	**13** (1.0)	--	--	--	**17** (1.5)	**11** (1.2)	<**0.001*****	63
Maize	**2.0** (0.3)	**2.1** (0.3)	--	ns	**2.6** (0.3)	**2.1** (0.4)	0.06 ns	21
Cereals ♣	--	**2.5** (0.2)	**1.2** (0.2)	<**0.001*****	**1.7** (0.2)	**1.8** (0.2)	ns	30
	N Export (kg·ha^{-1})							
Potato	**34** (2.6)	--	--	--	**41** (4.3)	**26** (2.2)	<0.01**	63
Maize	**46** (7.3)	**34** (7.3)	--	ns	**49** (5.6)	**30** (9.4)	0.10 ns	21
Cereals♣	--	**56** (5.0)	**24** (3.5)	**0.001*****	**41** (6.0)	**32** (2.9)	0.14 ns	30
	P Export (kg·ha^{-1})							
Potato	**6** (0.5)	--	--	--	7 (0.7)	**4** (0.3)	<**0.001** ***	63
Maize	**16** (2.2)	**10** (2.2)	--	**0.05** *	**18** (1.7)	**8** (2.8)	**0.01** **	21
Cereals♣	--	**11** (1.9)	**5** (1.4)	<**0.001*****	**9** (2.4)	**6** (1.2)	**0.05** *	30
	K Export (kg·ha^{-1})							
Potato	**60** (4.2)	--	--	--	**78** (6.0)	**48** (4.6)	<**0.001** ***	63
Maize	**91** (13.0)	**71** (13.0)	--	0.06ns	**101** (10.0)	**61** (16.8)	0.06 ns	21
Cereals♣	--	**56** (8.7)	**27** (6.4)	**0.02***	**43** (9.4)	**35** (6.1)	ns	30

♣ 'cereals' for yield are wheat and barley; for N, P, and K exports, cereals are wheat, barley and forage oats.

Yields and nutrient exports of crops varied widely, and were significantly different both between years in the rotation and between near and far fields. For example, fresh yields of potato varied 10-fold, from 3 $Mg \cdot ha^{-1}$ to over 30 $Mg \cdot ha^{-1}$, and outliers of maize grain yields showed a 100-fold variation from under 100 $kg \cdot ha^{-1}$ to over 5 $Mg \cdot ha^{-1}$. Extremes in yields and farmer explanation of these yields suggested that disease and weather events like hail or drought likely play significant roles in constraining crop yields. Nevertheless, a substantial amount of variation in crop yields and nutrient exports was due to differences between near and far fields, and also to apparent nutrient depletion in the course of the rotation (Table 3.5), suggesting that soil fertility was a strong driver of variation in yields and nutrient export variation. Cereal crop N, P, and K exports were lower in year three than year two of the rotation, and P export of maize crops was lower in years without manure application than those where manure was applied (Table 3.5).

Yields of potatoes and nutrient exports of several crops were higher in near fields than in far fields, consistent with the differences in manure application of nutrients presented above. These differences were especially strong in the case of potato yields, as well as P exports of all crops sampled (Table 3.5).

Erosion: strong driver of infield/outfield differences

Erosion rates measured on six cereal fields varied significantly with slope and slope length, allowing calibration of erosion to the topographic slope length and slope factor LS of the RUSLE (Fig. 3.2). Erosion measured on these fields ranged from 29 to 134 $Mg \cdot ha^{-1}$, with higher rates on steeper and longer slopes. Losses of total soil N and P on the steepest slopes were substantial, and moderate losses of exchangeable K were also measured. Coefficients determined for use in mass balances on sampled crop fields were [17.1, 8.0, 3.2] * LS for N, P, and K_{exch} losses respectively.

Table 3.5. Nutrient balance terms and balances for N, P, and K in near and far fields. All figures are annualized across the length of a rotation of either 2, 3, or 6 years. For purposes of comparison, balances are given without erosion and with N and K leaching and gas losses, with erosion and without these losses, and with all losses and erosion. Near fields are within 500m of the community, while far fields are >500m from the community.

	N, kg·ha^{-1} per year			P, kg·ha^{-1} per year			K, kg·ha^{-1} per year		
	Near	***Far***		***Near***	***Far***		***Near***	***Far***	
Manure in	**33.7** (4.5)	**17.1** (2.0)	***	**9.8** (1.6)	**4.4** (1.5)	***	**31.0** (5.1)	**10.6** (1.5)	***
N fixation in	**1.5** (0.5)	**3.2** (1.0)	ns	--	--		--	--	
Crop export out	**21.0** (2.7)	**10.1** (1.3)	***	**6.2** (1.4)	**2.8** (1.3)	***	**53.2** (4.8)	**21.8** (2.0)	***
Erosion out	**4.7** (1.9)	**11.8** (1.7)	*	**2.9** (1.1)	**6.9** (1.0)	*	**1.7** (0.7)	**3.9** (0.6)	***
Leaching and gaseous losses out♣	**12.0** (1.6)	**6.3** (0.8)	***	--	--		**5.4** (0.9)	**1.6** (0.8)	***
Balance with leaching/ gaseous losses, no erosion	**0.9** (2.5) 0	**3.3** (2.2) 0	ns	**3.6** (1.3) >0	**1.6** (1.2) 0	ns	**-22.2** (5.8) <0	**-11.3** (5.1) <0	ns
Balance with erosion, no leaching/gaseous losses	**7.2** (4.2) 0	**-5.8** (3.7) 0	***	**2.0** (1.8) 0	**-5.5** (1.7) <0		**-19.2** (6.2) <0	**-13.9** (5.5) <0	ns
Balance with all losses and erosion	**-6.2** (3.5) 0	**-12.7** (3.1) <0	**p= 0.08**	**2.0** (1.8) 0	**-5.5** (1.7) <0	*	**-23.9** (5.7) <0	**-15.2** (5.1) <0	ns

♣ Estimated using transfer functions in (Lesschen et al., 2007). Likely represents an upper bound on these losses because soluble fertilizer inputs are not used in these fields and because runoff of rainfall reduces lessens soil water available for leaching and denitrification. These losses are most realistic for flatter fields in this area that have N surpluses.

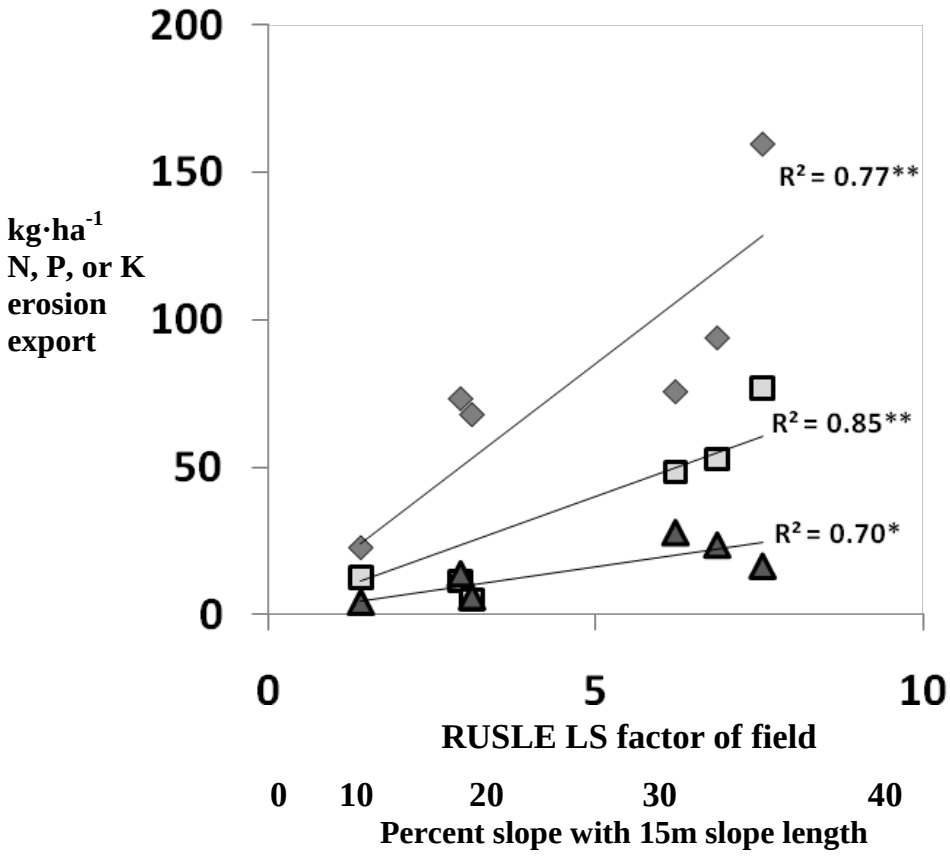

Figure 3.2. Measured soil erosion rates of N, P, and K regressed against RUSLE LS factor of six cropped fields in the study area. For a slope length of 15m typical for measured fields, the percent slope is also indicated below the horizontal axis. All fields were in wheat or barley for the year of measurement of denudation rates. Linear regressions shown were used to estimate soil erosion based on LS factors of cropped fields in nutrient balances.

Meanwhile, measured erosion on six fallow fields was more erratic, with one steep fallow field that had heavy animal traffic showing a high erosion rate of 121 $Mg \cdot ha^{-1}$, and another four sites showing a weak positive relationship between the LS factor and measured erosion, with a mean erosion rate of 8.4 $Mg \cdot ha^{-1}$. The final fallow site was discarded because data were compromised by excessive disturbance of the stakes by animals and people. For fallow fields, the slope-erosion relationship we fit to the LS factor was [2.4, 1.4, 0.26] * LS for N, P, and K_{exch}.

Because far fields were on average steeper than near fields, erosion losses of N, P, and K_{exch} were greater in far fields (Table 3.5). Slopes and LS factors for far vs. near fields were significantly different, with slopes 13 vs. 5.2 degrees ($p<0.01$ for t-test). The range of slopes was also greater for far than for near fields: 75% of near fields had slopes below 6%, while this third quartile level for far fields was higher at 23% slope. Far fields ranged widely in slopes from 1% to 30%: many occupied steep hillsides while others were in flatter hilltop positions.

Mass balances: infield/outfield distinctions

Mass balances showed two important results for annualized trends in nutrients. First, nutrient balances without erosion were balanced for N and P in far fields, and positive for P in near fields, while all K balances were in deficit due to crop exports (Table 3.5). Second, erosion was a large flow in comparison to manure inputs and crop exports, and larger erosion losses in far fields led to more negative trends for these fields.

In the case of near fields without erosion, P balances were significantly above zero, indicating that if erosion were reduced on these fields they would likely accumulate P. Zero and positive balances for N and P, in spite of nutrient inputs that were higher in near than in far fields, resulted from crop exports that were significantly correlated to total nutrient inputs, consistent with crop response to higher

fertility in infields ($p<0.001$ for N, P, and K exports regressed on manure inputs). Meanwhile, negative K balances, even without erosion, were due to the fact that K exports of all crops were moderate to high (Table 3.4, bottom) due to large amounts of plant biomass exported from fields: potatoes, grain straw, and maize stover. Uncertainties are larger for K inputs than for P in these estimates because of K in animal urine that may have entered the soil directly from corralling on fields. However, K deficits remained when low-elevation fields with corralling were removed from the analysis, or even when both manuring strategies were assigned the higher manure K content from high-elevation fields (data not shown), strengthening the conclusion that these rotations have persistent K deficits from crop exports.

The second major conclusion from balances is that when erosion was included, larger erosion losses in far fields vs. near ones created net negative N and P balances in far fields (Table 3.5, bottom). In the case of P balances, likely the most accurate balance of the three nutrients, negative balances in far fields suggests that larger overall P exports from erosion outstrip the smaller P inputs in these fields. Nevertheless, the large range in slopes among far fields indicates that it is likely slope and erosion rate, rather than distance from the community per se, which creates negative balances. Flat, far fields in fact had low erosion rates and P balances that were intermediate between steep, far fields and near fields. In summary both lower manuring rates and higher erosion rates in far fields caused the contrast between steep outfields with negative balances, and flat, well manured infields with positive P balances. Far/near contrasts in manure inputs and erosion losses also carried through to crop response and larger nutrient exports in near fields.

Scenarios for reversing soil fertility decline with P inputs and erosion management

Alternative scenarios for intensification in a representative far field indicate the additional manure and stress on rangeland resulting from intensification via shortened

Table 3.6. **Scenarios for net annualized nutrient balances over 18 years of rotation on a field with 10% slope**. Results shown are means and standard deviation of 16 runs of rotation with random draws for crop yields. The reference rotation repeats in six year cycles, with three years of cropping and three years of fallow, and manure once per cycle. 'Intensification' has shorter fallows, with potatoes 4 times in 18 years instead of three, and a 33% increase in manure application. Intensification with rock phosphate (RP) and legumes follows the same intensification pattern but has two years of green manure before potato, and RP addition to four legume crops in the rotation. The same pattern is followed in the last rotation, but with measures such as phalaris grass contour barriers and sediment capture trenches so that erosion is halved, with a consequent 5-10% loss in cropped area.

	Annualized balance, kg·ha^{-1}·y^{-1} (std deviation of 16 runs)			
Scenario	**N**	**P**	**K**	**Crop Rotation and fertility inputs** **Fallow and green manures shown in bold**
***1. Status quo* rotation**	**0.8** (1.8)	**-4.5** (0.5)	**-6.3** (3.1)	P-W-Fo-T-**ff**-P-M-Fo-**fff**-P-Fb-W-**fff** **Manure:** 410-85-390 kg·ha^{-1} N-P-K over 18 years
2. Intensification, shorter fallow	**1.4** (2.7)	**-4.5** (0.6)	**-5.1** (3.7)	P-W-Fo-**f**-P-M-Fo-**ff**-P-Fb-Fo-**ff**-P-W-T-**f** **Manure:** 520-115-510 kg·ha^{-1} N-P-K over 18 years
3. Intensification with RP addition	**5.6** (1.5)	**2.7** (0.8)	**-13.4** (4.9)	P-W-F/V$_{rp}$-**Gm**-P-M-T$_{rp}$-**ff**-P-Fb-F/V-**f**-**Gm**-P-W-T-**f** **Manure:** 410-85-390 kg·ha^{-1} N-P-K over 18 years **Rock phosphate:** 160 kg·ha^{-1} P as RP in y 3,4, 7, and 13
4. RP addition and erosion reduction measures	**10.6** (1.5)	**5.8** (0.8)	**-12.2** (4.9)	P-W-F/V$_{rp}$-**Gm**-P-M-T$_{rp}$-**ff**-P-Fb-F/V-**f**-**Gm**-P-W-T-**f** **Manure:** 410-85-390 kg·ha^{-1} N-P-K over 18 years **Rock phosphate:**160 kg·ha^{-1} P as RP in y 3,4, 7, and 13 **Erosion management** for 50% of *status quo* erosion

Crops in rotation: P, potato; W, wheat; Fo, forage oat; T, tarwi; M; maize; Fb, fava bean; F/V, forage oat with vetch; Gm, tarwi green manure; **f**, fallow

fallow lengths (Table 3.6). Alternative intensification strategies illustrate potential improvements in N and P status using practices such as rock phosphate addition, legumes, and erosion management. Using average rates of manure nutrient application measured in our sampling and similar crop sequences to those we observed, the status quo rotation shows similar N and P deficits to the far fields from sampled field budgets in Table 3.5. Intensification to shorter fallow lengths in scenario two, with use of two legume crops over 18 years, does not change these deficits markedly. It however requires 33% more manure, which would lead to increased stress on grazed rangeland, perhaps resulting eventually in reduced manuring rates as suggested by the significant correlation between rangeland NPP and manure inputs (Fig. 3.1).

In scenario three, several changes were made that make N and P balances positive. Legumes were intensified in the rotation in the form of two years of green manures prior to potatoes, so that manure was used at half the typical rate in these years and total manure use is no greater than the status quo amount. Vetch was also combined with forage oat crops to add additional fixed N to forage and spare this important nutrient from export. Rock phosphate was also added in substantial amounts over the eighteen years, with the idea of rehabilitating a depleted outfield for the longer term. The additional erosion management in scenario four doubled the improvements of scenario three on soil N and P stocks. Conserving nutrients through erosion control led to annual gains in N and P that were equal and opposite to negative trends in the status quo rotation. It should be noted though that as P and green manures were added in scenarios three and four, K deficits were worsened because manure K was reduced.

Discussion

Our study of nutrient balances in these extensive smallholder systems assessed the relative importance of wealth levels, management differences, and the impacts of erosion and rangeland productivity. Our discussion addresses the three main conclusions of our study. First, the lack of differences in fertility management based on wealth levels, and strong patterns in the way that crop rotation that utilizes peaks and troughs of fertility for different crops, indicate a conscious and structured management strategy that is common across different economic levels of farmers. Second, near/far differences in fields cause differences in productivity that are likely related to both conscious choices in management among fields and to shortening fallow lengths. Third, erosion and rangeland NPP are factors outside the crop management goals of these rotations that relate to yearly food production. The externality of erosion and rangeland productivity make them likely blind spots, factors that seem beyond the scope of management of these smallholders but that may be at the root of long-term degradation. Examining these factors better explains the constraints on soil sustainability of extensive agroecosystem managers in mountainous regions, moving beyond stereotypes of smallholders as either poverty-trapped degraders or monolithic guardians of soil sustainability.

Soil management strategies: evidence for robust community knowledge

Shared management strategies across wealth levels did not support our hypothesis that wealthier farmers would apply more nutrients to fields, and consistent patterns in different communities' crop rotations showed an attempt to use nutrients efficiently and create pulses of nutrients with manure when most needed by crops. Three results illustrate this: the first is the marked lack of difference among farmers at different wealth levels in nutrient application rates (Table 3.1). Amount of animals scaled with the amount of land, and coping mechanisms to reach acceptable manuring

levels exist even for the case where there are no animals. Second, balances for N and P were close to zero or positive in both far fields and near fields when erosion was ignored. In effect, crops exported most or all of the applied manure nutrients, in agreement with Couteaux' (2008) findings with a litterbag decomposition study for a potato-barley crop rotation on the Bolivian *altiplano*. Third, exports for several crops, especially small grains, showed a pattern of soil depletion with differences in exports between successive years (Table 3.4). Peaks and troughs of soil fertility were being induced by fairly large applications of manure nutrients to crops that most needed them. All of these features suggest an intentional approach to nutrient management that is shared across the community. This conclusion is in agreement with Pestalozzi, who described farmers' knowledge and intentional management of soil fertility regeneration in Andean sectoral fallows (2000) , and with Boesen and Friis-Hansen (2001), who challenged the idea of smallholders 'doing nothing' for soil fertility in Africa by describing multiple practices in use by smallholders aimed at managing soil fertility. The fact that neither fertility inputs nor total balances are stratified by wealth is in contrast to research in Zimbabwe, where wealthier farmers were found to use greater inputs on a per-area basis, especially of fertilizer (Cobo et al., 2009) or Ethiopia, where differences in nutrient balances among wealthy and poor farmers varied with elevation (Elias et al., 1998). In spite of large economic differences denoted by land area, herd sizes, and aggregate nutrient flows managed (Table 3.1), our results suggest a cohesive community sense of appropriate crop sequences and appropriate soil fertility management for growing these crops, even if formal communal arrangements of nine-year sectoral fallows described by Pestalozzi (2000) are in decline as survey and focus group data would suggest (Jones and Vanek, 2010; Sanchez, 2005) .

Contrasts between near and far fields

The large differences in nutrient inputs between near and far fields supported our hypotheses, and are consistent with the idea that in applying less manure farmers are responding to distance, slope of fields, and reliance on fallow. The relative ease of manuring fields close to a community, versus hauling it with pack animals over distances that ranged up to 1200 m on steep mountain slopes (the farthest distance manure was transported) is the simplest explanation for why rates would be lower on more distant fields. Also, farmers utilizing a far field after fallow are likely to credit nutrients in fallow vegetation, a point of farmer knowledge demonstrated by Pestalozzi (2000), and therefore use lower manure rates. However, since near fields were generally flatter and have lower erosion rates, it is also reasonable to think that farmers may invest greater nutrients in near fields, where the return in crop productivity over the long term is greater due to lower erosion rates. Higher manure application rates in flatter infields would reinforce contrasts in fertility over time.

Near/far distinctions in nutrient balances have been noted in other research, but they were especially strong for P balances in our study, a result that has practical implications. Elias et al. (1998) measured negative N balances for *shoka* outfields in Ethiopia (approximately -30 kg $N \cdot ha^{-1}$), in which crop exports alone were enough to drive balances negative. In contrast to our results, P balances of the Ethiopian outfields were positive, due to lower erosion losses than in our study and low crop exports which occurred because so little applied P was taken up by crops from P-fixing soils. By comparison, in our study near/far contrasts for P occurred in most of the mass balance terms: manure application rates, crop exports, crop nutrient contents, and whole balances with erosion. The fact that near/far distinction was so strong leads us to recommend that measures to address nutrient depletion such as erosion

management or use of additional P fertility sources should be especially focused on P in steep, far fields.

K balances and limitations of the budgeting approach

Negative K balances suggest that K depletion will occur over the long term in these systems. However, because the buffering potential of soil K is large in these soils K supply is less important to farmers in immediate management, and also less amenable to a budgeting approach. In contrast to P balances that differed for infields and outfields, K balances of fields were on average negative regardless of erosion or distance from the community due to large amounts of K export in tuber crops and residues from most harvests. Any K from animal urine omitted by sampling would reduce but not cancel this potential for K depletion: negative K balances occurred even when two best-case assumptions were made to boost K content of low-elevation manures in the set of balances (see *mass balance* results). To the extent that these farmers are managing for soil nutrient sufficiency, they seem to be focusing efforts on N and P fertility and ignoring K depletion. Farmers' practices are adapted to potassium's tendency to be highly buffered in soils, especially in younger soils with mineral clays such as those found in the area. This result also suggests that K nutrient balances are not predictive of short or medium term productivity in the case of K. Research from a 30-year cropping experiment in Finland supports this idea since rotations with no applied K showed a 20-year lag before yield declines began (Jaakkola and Yli-Halla, 2008). Also, new research on 2:1 clays like those in the younger soils from this area suggest that reserves of plant-available interlayer K in clays may be as large as 3 Mg $K \cdot ha^{-1}$ (Barre et al., 2007), far larger than the annual declines we document here. In far fields, acquisition of these reserves, and weathering of additional K from minerals, may be an important function of fallow phases. If productivity is sustainable in near fields with more continuous cropping, it might also

suggest other K inputs. Atmospheric deposition in dust is unlikely to significantly alter these negative trends, given maximum figure of 9.5 kg $K \cdot ha^{-1} \cdot y^{-1}$input for West African harmattan deposition from Lesschen et al. (2007), probably well above dust deposition rates in highland Bolivia. Flows such as human urine and excrement, wood ash, and other wastes around communities, might merit further research for near fields. Although farmer management is not currently responding to K limitation, depletion of this nutrient might be accentuated if productivity of these systems were increased through the use of green manures, as seen in our scenario analysis (Table 3.6). Potassium is known to be important for potatoes, and influences tolerance of crops to drought, frost, and disease which are common occurrences for smallholders without irrigation (Marschner, 1995). We analyzed17 field soils, for available K from around the study area showed that over half were in the 'low' and 'very low' categories for ammonium acetate- extractable K, which suggests that K limitation might be occurring in these systems and is worthy of more research.

Erosion: fulcrum point and blind spot for N and P soil fertility management

Our erosion measurements are consistent with direct observation of erosion in this system suggesting large losses of soil nutrients to erosion. They are reasonable outputs of the RUSLE model, assuming moderate erosivity of the climate and erodibility K values taken from RUSLE's erodibility nomograph, and a relatively high cover or C factor (i.e., low erosion protection) that produces high erosion rates from RUSLE (Renard et al., 1997). Our measurements are larger than soil losses for agriculture in tropical lowlands, e.g. 5-21 $Mg \cdot ha^{-1}$ in west African savannas (Pieri, 1989, slopes not given) and in good agreement with soil loss of 10-150 $Mg \cdot ha^{-1}$ modeled for sloped areas of a farmed watershed in Peru (Romero-Leon, 2005). The rates of soil denudation we measured, between 2 and 10 mm per year, are well within

the range of erosion rates reported for agriculture in a global literature review by Montgomery (2007).

Erosion is also a major fulcrum point for soil nutrient sustainability, as seen in the comparison of P balances of outfields with and without erosion, which seems to have real consequences for P exports of crops between near and far fields. P is especially vulnerable to erosion losses because it is hard to replace with amounts in manure that are smaller than inputs of manure N and K. This was so even with manure P contents in our study that were high compared to others: Elias et al. (1998) and Lesschen et al. (2007) for example document manure P contents from Africa about half those we report here, or about 2 $g \cdot kg^{-1}$ P. The importance of erosion in driving P depletion was also seen in a study from Ethiopia where erosion accounted for 80% of P losses and produced P deficits of -13 $kg \cdot ha^{-1}$ for smallholder systems, more negative than those we found here (Haileslassie et al., 2005).

Erosion is also a challenge that remains outside the reach of most farmers to effectively combat, beyond the use of contour tillage, diversion ditches above fields, and retaining walls or live barriers that are sometimes present in fields. It is interesting to compare partial attempts to manage erosion on steep fields with the communally shared and effective strategies at managing fertility of soils with manure we describe above. Both involve interaction of human managers with the environment: extraction of manure from rangeland to concentrate it on fields, versus intentionally modifying a challenging environment for farming so that erosion is reduced. Farmers acknowledge the problem of erosion. However fertility management with manure carries a shorter cause-effect cycle from manure application to crop production than management of erosion, where losses in productivity may not be immediately apparent within one or even a few years. Because erosion management is a longer term concern, and because shortages of both food and labor

are more immediate possibilities, management has a blind spot for erosion and tends to not fully address a serious problem of degradation. Of course, Andean farmers are tied by historical and cultural factors to mountain agroecosystems and manage within a marginalized economic context with erosion-prone land. Most Andean smallholders have limited options for risk reduction and investment in their systems. Referring to a 'blind spot' does not carry a moral judgment, but is meant rather to explain how the immediacy of crop management for subsistence, in addition to these other economic and cultural factors, function to strengthen patterns of degradation.

Rangeland NPP and long-term degradation

Rangeland productivity and grazing of rangelands to produce manure is another human-ecosystem linkage in many extensive cropping systems that is external to immediate goals of food production, and may therefore suffer particular pressure for degradation. Our finding that lower rangeland NPP is associated with smaller manure application rates and manure nutrient contents hints at a degradation trajectory, in which communities with degraded rangeland encounter difficulties in supplying enough manure to crops compared to communities with more productive grazing resources. The pressure to increase manure production to substitute for natural soil regeneration as fallow lengths are shortened could provide a tipping point in a trajectory toward degraded rangelands. Similar to soil erosion, rangeland degradation is not as immediately apparent in its impacts on crop production and is a blind spot compared to the yearly need for manure applications. Additionally, reduced rangeland productivity could in fact accelerate degradation of cropped fields due to lowered amounts of manure and increasingly larger deficits compared to erosion.

There are alternate explanations besides a degradation trajectory to explain differences in NPP and manure application rates. Soil, microclimate, and topographic differences could produce variability in NPP based on different adapted rangeland

species, growth rates, and prevalence of N fixers. The total area of rangeland available to a community, degree of communal or private ownership, and whether animals can access it easily due to topography would also influence manure nutrient flows and crop production. It would be important to gather more data from a larger set of extensive farming systems that use rangeland as a source of manure fertility to test whether NPP relates in a consistent way to manure application. It would also be important to test whether NPP has changed over time in ways that affect farmers' ability to supply nutrients to crops, both of which are reasonable hypotheses.

Scenarios and impacts of changes

Our scenarios demonstrate ways that one of the most nutrient-stressed components of the cropping system – a sloped outfield with moderate rates of erosion and low rates of manure inputs – could be rehabilitated using rock phosphate and legumes (Table 3.6). Scenario 4 for this field restates the importance of addressing erosion, and legumes are shown to have dramatic potential for impact for increasing N stocks and forage quality, and substituting for manure in major crop production years. Of course, farmers' focus on immediate crop production concerns and their well-tried current knowledge system of soil fertility mieght make promoting this level of investment in a field challenging. Regardless of challenges inherent to adoption of any innovation, however, these scenarios synthesize a great deal of research by ourselves and others and have potential to strengthen a wide range of measures to reverse degradation. Soil erosion is likely the most important problem to tackle for longer term impacts, in conjunction with other changes in nutrient management that might be proposed. Positive short-term outcomes could also be used to 'market' changes in practice to farmers: green manures reduce the carrying of manure and frees manure for use on other fields; vetch improves the forage quality of oats; rock phosphate improves yields of tarwi for consumption and earnings.

Conclusions

Our study tested how management, wealth levels of farmers, and environment impact trends for soil nutrient sustainability in an extensive smallholder system, as a way of enriching the view of smallholder farmers as either poverty-trapped degraders or romanticized sustainers of their environments. In this extensive mountain ecosystem, sustainability depended relatively little on wealth levels of farmers because of strategies for management that were shared community-wide. These strategies for soil nutrient management were structured to utilize ebbs and flows of nutrients with different crops through the rotation, and were successful in producing sufficient crops in good years, with scarcity that likely affects poorer farmers, more from lack of land than from deficient nutrient management. If erosion rates were lowered, these strategies would also be sustainable due to balanced or positive nutrient mass balances. However, environmental factors such as erosion and rangeland carrying capacity create unsustainable trends that are acting external to the yearly cycles of cropping and nutrient management. There was a strong role played by erosion in driving balances negative on steeper fields, and data that suggests that rangeland resources are being degraded and in turn affect manure inputs. So, beyond the horizon of year to year food sustenance, these farmers have not been able to address longer-term degradation. To respond to erosion and rangeland degradation, the existence of community-wide strategies of nutrient management suggests that successful approaches can come from fostering community-wide attitudes and decisions and proposing new measures across different economic levels within the community. Several scenarios demonstrate that negative trends in soil nutrients could in fact be reversed, especially through the control of erosion and a focus on depleted outfields.

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